The use of phosphoric acid as an alternative phosphorus source in broiler chicken production

By

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Declaration

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General abstract

In the broiler industry, it has become common practice to supplement diets with feed grade phosphates and in doing so provide sufficient available phosphorus to meet the birds' requirements. Supplemented phosphates provide a large portion of the total available phosphorus within the diet and any small differences in the availability could have significant effects on whether or not the bird meets its nutritional requirements for phosphorus. If the bird's phosphorus requirements are not met, the implications are detrimental, not only to the bird but also to the farmers' flock productivity. Two experiments were conducted; the objectives of the first experiment were to assess the phosphorus bioavailability and nutrient and mineral coefficient of total tract digestibility (CTTD) of a feed grade inorganic phosphate source, defluorinated phosphoric acid by means of a digestibility study. Five treatments were used in this trial. Two diets made the basis of the treatments, a summit diet, only supplemented with mono-dicalcium phosphate (MDCP), and a dilution diet, only supplemented with phosphoric acid. These were mixed in ratios of 100:0, 75:25, 50:50, 25:75: and 0:100 respectively. The second experiment assessed the effect of supplementing broiler diets with either defluorinated phosphoric acid or defluorinated and desulfonated phosphoric acid. The control diet used was supplemented with MDCP. The phosphoric acids were included in the diets at two inclusion levels, based on dietary total phosphorus levels and available phosphorus levels. Furthermore, during the mixing of the diets, the phosphoric acids were either added to the diets' grain component (maize, soybean 46 and full fat soya meal) before any of the remaining macro minerals were added or added to the diet last after all the other ingredients had been sufficiently incorporated. These treatment differences resulted in eight test dietary treatments. The objectives for this experiment were threefold: (i) to evaluate the effects of the dietary treatments on production parameters; (ii) to determine the influence of the dietary treatments on carcass characteristics and meat quality and (iii) to investigate the effect of the dietary treatments on broiler organ weights, intestinal pH and bone parameters. The phosphorus bioavailability of the phosphoric acid (PA) showed to be greatest and revealed high digestibility values of all nutrients and minerals. For the second experiment, both PAs resulted in ideal measurements of the production parameters (live weight, cumulative weight gain, cumulative intake, feed conversion ratio, average daily gain, European production efficiency factor and protein efficiency ratio) regardless of when the PA was added to the diet during mixing. However, when acid inclusion levels were based on the diet's total phosphorus, these values decreased significantly irrespective of the method of mixing. Liveability was also affected by treatment differences, as a result of sodium poisoning in the control diet on day 14 for approximately 26 hours, and P deprivation

in the final week of the trial. Concerning the carcass characteristics and meat quality, carcass portion weights of the thigh, drumstick and wing, as well as the breast colour CIE-Lab L*, a* and b* measurements together with the chroma values showed differences. Hue angle was not significantly affected by the treatment differences. No obvious indication was found for the cause of the portion weight difference, and the breast colour, although different, was found to be normal for all treatments. Bone breakage strength was affected by dietary treatment differences. The birds that were fed diets with sufficient PA inclusion levels, based on the dietary available phosphorus, had significantly greater breakage strength and were not affected by the different mixing methods used between treatments. Organ weights relative to body weight, tibia calcium and phosphorus content and the pH reading of the small intestine, except the cecum, were not influenced by the dietary treatment differences. The cecum from birds of all the treatments was found to be more acidic than normal. Overall, the results show PA to be a highly available phosphorus supplement in broiler diets with competitive CTTD values. Furthermore, PA's were not the cause of any negative effects on growth performance, carcass characteristics, meat quality, gut and bone parameters. Therefore, these two inorganic phosphate sources are ideal sources of phosphorus and can be used in broiler diets to ensure growth and production are maintained as expected with no adverse effects on the bird's health.

Algemene opsomming

In die braaikuiken industrie is dit algemene praktyk om voere met voergraad fosfate aan te vul ten einde voldoende fosfor (P) te voorsien om aan die diere se behoefte te voldoen. Hierdie aanvullings voorsien die grootste gedeelte van die totale beskikbare P in die dieet en 'n klein verandering in die beskikbaarheid kan bepalend wees of daar aan die P behoeftes voldoen word al dan nie. Indien daar nie aan die behoeftes voldoen word nie is die gevolge nadelig, nie net vir die dier nie maar ook vir die effektiwiteit van die produsent.

Twee proewe is uitgevoer; die doelwitte van die eerste proef was om die P biobeskikbaarheid en nutriënt- en minerale verteerbaarheid van voergraad anorganiese fosfaatbronne en defluorineerde fosforsuur (PA) te bepaal deur middel van 'n verteringsproef. Die proef het uit vyf behandelings bestaan. Twee diëte is gemeng as die basis vir die behandelings, 'n maksimum insluitingsdieet en 'n verdunningsdieet. Die maksimum insluitingsdieet het slegs mono-dikalsium fosfaat (MDCP) bevat terwyl die verdunningsdieet slegs PA bevat het as P bron. Hierdie twee diëte is vermeng in 'n verhouding van 100:0, 75:25, 50:50, 25:75: en 0:100. Die tweede proef het ten doel gehad om die invloed van die gebruik van gedefluorineerde PA en gedefluorineerde en gedesulfoneerde PA op die produksieparameters en vleiskwaliteitseienskappe van braaikuikens te bepaal. Die PA'e is ingesluit by twee insluitingspeile gebaseer op die totale (tP) en beskikbare P (aP) vlakke. Verder is die vermenging van die PA'e gevariëer. In die een geval is die PA gemeng met die graan komponent (mielies, soja oliekoekmeel en volvet soja) voordat die res van die voormengsel bygevoeg is of die PA'e is toegevoeg aan die einde van die mengproses nadat alle ander bestanddele reeds toegevoeg is. Hierdie het tot agt behandelings aanleiding gegee. Die doelwit van die proef was drievoudig: (i) om die invloed van die behandelings op produksieparameters te bepaal; (ii) om die invloed van die behandelings op karkas- en vleiskwaliteit eienskappe te bepaal en (iii) om die invloed van die behandelings op orgaanmassa, ingewands pH en beenparameters te bepaal. Die P-beskikbaarheid van die gedefluorineerde PA was die hoogste en het ook aanleiding gegee tot die hoogste verteerbaarheidswaardes vir alle ander nutriënte en minerale. Beide PA'e het aanleiding gegee tot ideale produksie parameters (lewende massa, massa toename, kumulatiewe voerinname, voeromsetverhouding, gemiddelde daaglikse toename, Europese produksie effektiwiteit faktor en proteïen effektiwiteit verhouding), hierdie resultaat was onverwant aan die mengprosedure. Wanneer insluitingspeile van P gebaseer was op totale P het hierdie waardes aansienlik verswak en oorlewingstempo was ook negatief beïnvloed. Die verswakte oorlewing was eerstens as gevolg van

die oorvoorsiening van Na vir ongeveer 26 uur vanaf dag 14 en tweedens as gevolg van P tekorte tydens die laaste week van die proef. Karkaseienskappe en vleiskwaliteit (porsie massa, borsvleis kleur) is deur behandeling beïnvloed maar die rede hiervoor was nie ooglopend nie. Alle kleur metings was normaal vir alle behandelings en verskille wat gemeet is, was klein en verslille is waarskynlik nie met die blote oog waarneembaar nie. Beensterkte is deur behandeling beïnvloed. Die kuikens wat voer ontvang het volgens aP het sterker bene gehad as die wat voer volgens tP ontvang het. Die mengmetode het geen invloed op beensterkte gehad nie. Orgaanmassa uitgedruk as verhouding tot liggaamsmassa, tibia Ca en P inhoud en pH van die dunderm is nie deur behandeling beïnvloed nie. Samevattend kan dit gestel word dat die gebruik van PA ’n hoogs beskikbare P bron vir braaikuikens is met geen negatiewe uitwerking op produksieparameters, karkas- of vleiskwaliteit eienskappe, SVK of been parameters nie.

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Notes

The language and style used in this thesis are in accordance with the requirements of the South African Journal of Animal Science with changes to improve readability. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters is therefore unavoidable.

Abbreviations

ADG	Average daily gain
AME	Apparent metabolisable energy
ANOVA	Analysis of variance
Al	Aluminium
aP	Available phosphorus
Bo	Boron
Ca	Calcium
Cu	Copper
DF	Defluorinated
DFS	Defluorinated and desulfonated
EPEF	European production efficiency factor
FCR	Feed conversion ratio
Fe	Iron
g	Grams
h	Hours
iP	Inorganic phosphate
K	Potassium
kg	Kilograms
kPa	Kilopascals
M	Mol
M	Metres
Mg	Magnesium
mg/g	Milligrams per gram
ml	Mili litres
mm	Mili metres
Mn	Manganese
N	Newton
Na	Sodium
N/g	Newton per gram
npP	Non-phytate phosphorus
P	Phosphorus
PA	Phosphoric acid
PER	Protein efficiency ratio
pH_i	Initial pH (15 minutes *post mortem*)
pH_u	Ultimate pH (24 hours *post mortem*)
tP	Total phosphorus
Zn	Zinc

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Chapter 1

General Introduction

The South African poultry industry contributes the largest value to the country's total gross agricultural production and provides the country's customer with a highly affordable protein source (Bureau for Food and Agricultural Policy & National Agrucultural Marketing Council, 2016). To cope with the demands of the market for affordable protein, the modern broiler is reaching marketable weight earlier and earlier (Kleyn & Chystal, 2014; Beski *et al.*, 2015). The need to meet the ever-developing broiler requirements places nutritional advances at the forefront of development so as to ensure the modern broiler's growth is achieved and to maintain sustainable broiler production. Therefore, diet formulation requires sufficient knowledge of the raw ingredients intended to be used, such that the birds receive maximum nutrient availability (Beski *et al.*, 2015).

Poultry diets are predominantly plant based with a shift away from animal protein sources. However, animal protein sources are known to have a high mineral availability, whereas plant sources have minerals which are predominantly bound in the form of phytate, causing them to be less readily available to monogastric animals (Driver, 2004; Rodehutscord, 2013). This gives rise to supplementing diets with inorganic mineral sources. Phosphorus (P) is one of the essential minerals required for sufficient growth and development due to its role in skeletal development as well as several metabolic processes (Suttle, 2010). Therefore, nutritionists must ensure the dietary requirements for P are met. However, due to the uncertainty around the availability of the P from different inorganic phosphate (iP) sources as well as the implications of undersupplying P, diets have been oversupplied with iP (Viljoen, 2001). This has led to excess P being excreted, resulting in potential environmental pollution of the soils and ground water (Waldroup, 1999) as well as an unnecessary waste of money, as dietary supplements have a tendency to be expensive. Interactions between different minerals which cause a reduction in the availability of the minerals within the diet have also been a cause for the oversupply. Minerals and their requirements were initially focused on one at a time, and this has been proven to be incorrect as mineral interactions have been reported (Nugara & Edwards, 1963; Davies & Reid, 1979; Henry & Miles, 2000). It now has been accepted that to obtain a

desired response, mineral concentrations need to be included to the diet according to the animal's requirements as well as in relation to other minerals (Hemati Matin et al., 2013).

The main objective in optimizing dietary P levels is to avoid deficiencies so as to maintain animal health and performance (Rodehutscord, 2013); a drop in P intake has negative effects on the growth and welfare of a growing bird (Driver *et al.*, 2005). In order to avoid the negative effects of P deficiencies, knowledge of the availability of the P and the P levels in the iP source is important. The most commonly used iP sources within South Africa are calcium phosphates. These are manufactured through reacting calcium salts with phosphoric acid (PA) (Viljoen, 2001), to produce mono-, di- or mono-dicalcium phosphates with P bio-availabilities of 84, 77 and 79%, respectively (Viljoen, 2001). Indications are that these iP sources are becoming scarcer and alternative P sources are being sought; phosphoric acid (PA) has been identified as being such a potential source of P suitable for inclusion in broiler diets. Yet very little is known about the potential of PA in monogastric animals' diets. Therefore, the aim of this study was to investigate the potential of PA as an inorganic P source on broiler production. Specific study objectives included:

i. To evaluate the phosphorus bioavailability and nutrient coefficient of total tract digestibility of defluorinated PA.
ii. To assess the production performance of broiler chickens fed a diet supplemented with defluorinated PA and defluorinated and desulfonated PA.
iii. To assess the effect of defluorinated PA and defluorinated and desulfonated PA on carcass characteristics and meat quality.
iv. To assess the effect of defluorinated PA and defluorinated and desulfonated PA on organ, gut and bone parameters of broiler chickens.
v. To determine whether the inclusion levels of the PA, based on the dietary total or available phosphorus levels, have an effect on the parameters mentioned in objectives ii, iii and iv.
vi. To determine whether the mixing sequence used when adding the PA to the feed influences any of the parameters mentioned in objectives ii, iii and iv.

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Chapter 2

Literature Review

2.1 Introduction

Living organisms rely heavily on phosphorus (P) to ensure everyday processes within the body run smoothly. Phosphorus not only is a major component of the skeleton, but its involvement in every phase of metabolism is of great importance, due to P being an essential component within organic compounds (Soares, 1995). Within the broiler industry, the major portion of phosphorus fed to chickens is supplied through plants and fishmeal. However, lately more information has been brought to light on the use of inorganic feed phosphates and their importance as a feed component is growing worldwide. The use of inorganic phosphates (iP) has come about as much of plant P is bound to phytate, which makes the P highly unavailable to monogastric animals (Van der Klis & Versteegh, 1999). On the other hand, P from inorganic sources is of high availability, leading to broiler diets being supplemented with these inorganic phosphates to meet the birds' requirements (Viljoen, 2001).

Phosphorus is essential to normal growth of broilers and due to the birds ever increasing demands for proper skeletal growth and body maintenance, an oversupply of P within diets has become the norm (Waldroup, 1999). This is to avoid the consequences of undersupplying P, namely; poor performance in growth, poor skeletal development which effects carcass quality, and high mortalities (Waldroup, 1999). An oversupply of P leads to lowering absorption efficiency of P from the gastrointestinal tract, kidney P excretion increases and faecal P levels increases. Furthermore, excessive excretion of P has been found to pollute soils and water systems; the later causing eutrophication (De Groote & Huyghebaert, 1997). Therefore, there is a need to minimize the levels of excreted P from animals. This can be achieved by supplying adequate amounts to meet the requirements of the animal and no more. In addition to the environmental effects of excessive use of inorganic phosphate sources, the global phosphate resources needed for the production of feed phosphates are diminishing (Rodehutscord, 2013). This makes handling and use of the inorganic P sources a vital focus in ensuring a sustainable agricultural industry.

Supplying diets with adequate P requires knowledge of the source's P availability; inorganic feed phosphates are not equally available due to the different biological values of these phosphates. The biological value is indicative of the amount of P which can be utilized within the phosphate (Waldroup, 1999; Rodehutscord, 2013), and further on will be referred to as the bioavailability. A number of methods have been employed as measurements of P bioavailability; these are quantitative measures, qualitative measures and *in vivo* measures (Shastak & Rodehutscord, 2013).

The purpose of this review is to give a better understanding of P and its involvement in growth and development of broiler chickens. Different methods of P bioavailability determination are discussed and factors that play a role in P availability as a whole are also elucidated on.

2.2 Physiology

Phosphorus (P) is one of the essential minerals required by broilers and is the second most abundant mineral in the body, second only to calcium (Ca). However, P fulfils most known functions within the body, from structural to metabolic functions (Soares, 1995), namely:

- Plays a large part in skeletal development and maintenance, as the skeleton houses the largest concentration of P in the body (80%). It is also a storage depot for Ca and P (Soares, 1995).
- Plays a role in cell wall formation by being an integral part of the phospholipids (Soares, 1995).
- Can be found involved in processes of energy storage and metabolism. Certain phosphates, for example adenosine diphosphate (ADP) and adenosine triphosphate (ATP), are found within the muscle cells and play a role in energy storage and utilization (Driver, 2004).
- Plays a role in the acid-base balance of the body, as well as the pH balance (Miles & Henry, 1997).
- It has involvement in cell growth and differentiation. Phosphorus is found in the structure of nucleic acids (Soares, 1995).
- Plays a role in voluntary intake and feed utilization efficiency in chickens (Bar & Hurwitz, 1984).

In the chicken, the gizzard is where available P is solubilised and becomes available, as orthophosphate, for digestion. The duodenum and jejunum is where absorption of P occurs in

the digestive tract, as the pH and absorptive capacity of the intestinal mucosa is ideal for such. However, absorption is not isolated to only these two compartments; absorption still takes place after the duodenum and jejunum although at a lower rate, as the rate of passage of the digesta is mostly too fast for thorough absorption. Furthermore, the control over the absorption of available P, when compared to the other minerals, is limited in birds (Hegsted, 1973). The P content of the body is regulated through the urinary system (Leske & Coon, 2002). Phosphorus excretion generally occurs when daily P intake exceeds what is required to support a bird's steady physiological state (Leske & Coon, 2002). Various factors pay a role in P absorption. These include the P and Ca levels in the diet, levels of vitamin D, P source, the Ca: P ratio, intestinal pH and the antagonistic effects of other minerals such as Zn and Cu (Soares, 1995; McDonald *et al.*, 1995; Van der Klis & Versteegh, 1999). The amount of phytase present in the bird also plays an important role in P requirements and absorption (Viljoen, 2001; Payne, 2005; Kleyn, 2013; Rodehutscord, 2013; Franklin, 2015). The role of phytase is discussed in section 2.6.5.

Phosphorus together with Ca are the two most important minerals to a chicken (Kleyn, 2013). It is for this reason, as well as their interactions with one another before and after absorption (Soares, 1995) and the fact that excessive quantities of one leads to poor use of the other (Van der Klis & Versteegh, 1999), that one cannot consider or discuss the one fully without mention of the other.

An imbalanced Ca: P ratio is known to limit the use of P (Mc Donald *et al.*, 1997). High levels of Ca is said to reduce the availability of P as it is excreted in the form of calcium phosphate (Ca-P complex) and this can lead to P deficiencies if critical levels are reached (Harrold *et al.*, 1983; Kleyn, 2013). Poor P utilization by birds due to adverse ratios of Ca and P have been attributed to:

- Reduced P absorption at high levels of Ca as the Ca-P complex is excreted.
- Excretion of absorbed P when Ca levels are low (Van der Klis & Versteegh, 1999).

The first symptoms of a P deficiency can be seen in young birds where the skeleton develops abnormally (Kleyn, 2013). The initial results of deficiencies in P is a drop in the phosphate levels of the blood plasma. Anselme (2003) and Kleyn (2013) speak of further P deficiencies causing loss of appetite, weakness and even death. Under close *post mortem* examination, the

ribs are deformed and the larger bones such as the tibia and femur are rubbery and easily broken: this is a condition known as rickets (Kleyn, 2013). Other symptoms of deficiencies include tibial dyschondroplasia and osteomalacia (Waldroup, 1999; Anselme, 2003).

2.3 Broiler Requirements

Phosphorus requirements in broilers are discussed in concurrence with calcium (Ca), due to the interactions between the two within a diet. As mentioned, any deficiencies or oversupply of one in the body will lead to a under or oversupply of the other (Driver, 2004).

The mineral requirements of chickens have generally been calculated using the factorial approach, which takes the birds maintenance and production requirements, age, genotype and performance level into consideration (Mc Donald *et al.*, 2011). In poultry, P requirements are centred on the intake of non-phytate phosphorus (npP) and this is expressed as available P (Driver, 2004). However, this assumption is incorrect as npP does not take into account the fact that not all npP is completely available and also that some of the phytate bound phosphorus can be utilized to meet P requirements (Leske & Coon, 2002). Table 2.1 shows the requirements of Ca and inorganic P for broilers.

Table 2.1 Inorganic Phosphorus and Calcium Requirements for Broilers (National Research Council, 1994)

Age (weeks)	Inorganic Phosphorus (%)	Calcium (%)
0-3	0.45	1.00
4-6	0.35	0.9
7-8	0.3	0.8

Numerous research findings exist from which these recommendations are based on however, most of the research findings were published between 1952 and 1983. The modern commercial broiler has changed dramatically from that for which these recommendations were made with special reference to growth, utilization of nutrients, feed conversion ratio, structural bone characteristics and carcass characteristics (Dhandu & Angel, 2003). This is due to changes in management practices, changes related to the diet as well as genetic selection (Havenstein *et*

al., 1994; Williams *et al.*, 2000). Havenstein *et al.* (1994) concluded that advances in genetics is the reason for most of the variation between the old and modern bird, followed closely by changes in the diets and their composition. More specifically, the modern bird has a greater feed utilization efficiency and diets are formulated to meet the fast growing birds' requirements. A more recent publication by Angel (2011) seems to be a better representation of the requirements of the modern bird in terms of calcium and available phosphorus (Table 2.2). Differences which stand out between the two tables are the ratio of Ca to P. Table 2.1 has a Ca:P ratio of 2.2:1 in the first 3 weeks of age where Table 2.2 illustrates a ratio of 2.9-3.6:1 during the same period. This change is due to nutritionists having a better understanding of broiler Ca and P requirements (Angel, 2011).

Table 2.2 Range of phosphorus and calcium requirements in modern broiler diets (Angel, 2011)

Age (days)	Ca (g/kg)	Available P (g/kg)
1-21 days	6.1-13.1	1.7-4.5
22-42 days	6.9-9.1	1.5-4.5

Phosphorus requirements have a close correlation with both Ca and vitamin D. As mentioned by Van der Klis & Versteegh (1999), an excess or deficiency in Ca will result in an abnormal Ca: P ratio, which can lead to reduced P absorption or excretion of absorbed P respectively. Vitamin D also plays a role in P requirements. Garcia *et al.* (2013) states that "vitamin D is required for the absorption of calcium and phosphorus in the intestines, increasing its utilization efficiency and consequently increasing bone ash content." It is therefore clear that in addition to the ideal Ca: P ratio and sufficient P levels, the levels of vitamin D is also essential towards determining P requirements in poultry (Mc Donald *et al.*, 1997).

2.4 Phosphorus Availability

2.4.1 Definition of Terms

Bioavailability is said to describe a raw material's potential (Rodehutscord, 2013). However, in past years, there have been a number of interpretations of the definition of available P (Shastak & Rodehutscord, 2013). Rodehutscord (2013) defines available P as the amount of P

used to meet the animal's requirements relative to the total dietary P, whereas other interpretations define it in terms of the form in which P is bound. For example, the National Research Council (1994) defined available P as the amount of non-phytate P within a diet. Angel (2011) defines available P (aP) as the amount of P absorbed by the animal from the diet and this definition is consistent with that of Rodehutscord (2013).

A number of available methods, which are used to determine the "bio"availability estimate, has given rise to many different definitions said to explain phosphorus utilization. The most widely used definition explains a nutrient's bioavailability as the amount of intake which the intestine is able to absorbed and make available for use within the body (Gueguen, 1994).

2.4.2 Evaluation of bioavailability

Due to variations in bioavailability of phosphorus within poultry feeds, there have been a number of methods developed to quantify phosphorus availability. These are divided into three groups, namely: qualitative measurements, quantitative measurements and *in vitro* tests. (Shastak & Rodehutscord, 2013).

2.4.3 Qualitative measurements

Qualitative measurements of bio-available P include bone ash content, bone breakage strength, blood P content and growth assays (Shastak & Rodehutscord, 2013). These bioassays only indicate a relative biological value and so cannot provide a measure of true P bioavailability. Furthermore, as these are qualitative measures they do not provide a quantitative measure of P bioavailability and so are limited in terms of value when performing diet formulations (Lima *et al.*, 1997).

2.4.3.1 Bone ash content

The bone is a strong indicator for P bioavailability as ~80% of the total retained P is located within the skeleton. The remaining 20% is said to be found within the bird's tissues (De Groote & Huyghebaert, 1997). For many years the testing of bioavailability has been focused on bone ash content (Shastak & Rodehutscord, 2013). Heuser & Norris (1926), who used Ca and P content of the tibia bone ash as a gauge of bone mineralisation, performed one of the first such studies. Nelson and Walker (1964) reported bone ash content as an accurate measurement of

dietary P levels. In addition, a later study by Nelson (1967) stated that one of the most sensitive methods of P evaluation was that of bone ash content.

Much literature has been published over time which identifies bone ash content as a strong method for estimation of P bioavailability; see for example Gillis *et al.* (1954), Hurwitz, (1964) or Lima *et al.* (1997). Gillis *et al*. (1954) performed one of the first studies to use tibia ash concentration to quantify P availability for chickens. Comparing the tibia ash percentage from chickens fed a given test P source to that of chickens fed a reference P source (beta-tricalcium phosphorus) they were able to gain a relative biological value of the test source. Hurwitz (1964), based on the regression of tibia P as a function of total P intake in growing chicks, was able to find a constant ratio between tibia P and whole carcass P, opening up the idea that tibia P could prove to be a good indication of whole carcass P content. However, the sensitivity of using the tibia over the femur has been questioned (Moran & Todd, 1994; Angel *et al.*, 2006). Furthermore, it has been found that obtaining ash content from the tibia and femur is laborious in that the cleaning of the bones is tedious and the fat extraction period is rather lengthy (Shastak & Rodehutscord, 2013). A much earlier study by Baird & MacMillan (1942) proposed the use of toe ash instead of tibia ash as a response criterion in the evaluation of calcification of Vitamin D. They found that the use of toe ash reduced the labour and time spent cleaning bones. Furthermore, there was no need to sacrifice the birds. Yoshida & Hoshii (1977) used toe ash as a determinant for available P and reported it to be as sensitive in assessing P bioavailability as when using tibia ash.

The length which an experiment is carried out for is said to effect bone ash studies (Shastak, 2012; Shastak & Rodehutscord, 2013). The first to find observations where bone ash was effected by the experiment duration was Ammerman *et al.* (1961). Using a degerminated corn-soybean meal diet, they evaluated P supplements in 10 and 28-day bioassays. Their results showed that the 28-day bioassay had greater sensitivity when obtaining data for a tibia ash response curve, than that of the 10-day bioassay. Other aspects, which might affect the bioavailability of an individual sample, is the phosphate reference, response criteria and the diets' Ca levels.

2.4.3.2 Bone breakage strength

The strength of bone is said to be the bone's ability to handle stress before it actually breaks (Rath *et al.*, 2000). Bone strength can be affected through various factors (Figure 2.1). The

nutritional effect is said to be the greatest influence on bone strength, particularly dietary Ca and P concentrations, where imbalances of either nutrient will lead to skeletal weaknesses (Van der Klis & Versteegh, 1999; Rath *et al.*, 2000; Li *et al.*, 2016). The reason for this being they form 95% of the mineral matrices (Rath *et al.*, 2000).

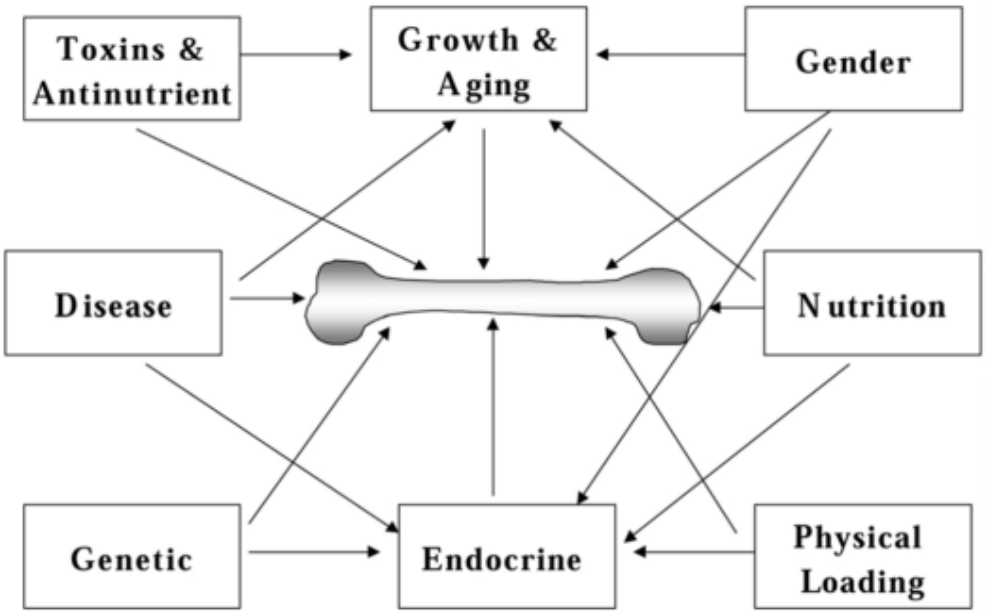

Figure 2.1 Effectors of bone strength (Rath *et al.*, 2000).

Research has shown that the use of bone breakage strength as reference for Ca and P content was first done by Rowland *et al.* (1967) (as cited by Shastak & Rodehutscord, 2013). They concluded that bone breakage strength was as good an indicator of P bioavailability as the use of bone ash content. However, Huyghebaert *et al.* (1980) and Shastak *et al.* (2012) found bone breakage strength to display poor sensitivity compared to that of bone ash content. Orban *et al.* (1993) also found that bone breakage strength may be affected by preparation differences of the bones, mechanical and physical properties of the bone and differences in the instrument used to record the values of bone breakage strength.

2.4.3.3 Blood

Various studies have been documented where inorganic P concentration in blood serum was used in testing leg weakness and to compare rachitic chicks to normal chicks (Hart *et al.*, 1922; Ackerson *et al.*, 1924). These investigations however did not test the actual relation between

the levels of P concentration in the blood to that of the diet concentration. Gardiner first did this in 1962. In these experiments, he found there was a linear relationship between the levels of plasma inorganic P and dietary inorganic P supplementation. A further publication two years later indicates that plasma inorganic P can be used as a measure for relative P availability (Hurwitz, 1964). Bansal (1990) concluded that although serum inorganic P constitutes a very small percentage of the total P in the body, it does give an indication as to the chickens' phosphorus status. A report by Cupisti & Kalantar-Zadeh (2013) found normal levels of P in the plasma to be 2.5-4.5 mg/dl, and these yields are usually the same as that found in the serum. Lima *et al*. (1997) experimented with three different levels of phosphorus supplemented diets (0.1, 0.2, and 0.3%) and the basal diet was formulated to have all the required nutrients except for phosphorus. They were able to conclude a distinct increase in plasma P as the level of supplementation increased.

2.4.3.4 Growth

Broilers (particularly modern broiler lines) tend to be more prone to variations in performance through changes in mineral supply as their mineral body reserves are low and their growth rates are high (Shastak & Rodehutscord, 2013). Therefore, use of growth or body weight to obtain the bioavailability of different phosphorus sources is possible. A number of studies have been conducted in the past years where body weight and bone ash, be it tibia or toe ash, were used to find the P and Ca bioavailability (Buckner *et al.*, 1930; Gillis *et al.*, 1948; Summers *et al.*, 1959; Vandepopuliere *et al.*, 1961; Huyghebaert *et al.*, 1980). Huyghebaert *et al.* (1980) experimented with different P sources to find their relative biological value (bioavailability). The criterion used to assess the bioavailability was live weight gain, feed conversion ratio, and bone breaking strength, bone ash percentage and bone ash P content. Controlled feeding ensured that all chickens had similar average intake throughout the experiment. This ensured that the effect of organic P was consistent for all the groups, therefore, any changes in these criteria, with an increase in P supplementation, relate to the supplemented P only. In their conclusion, they found there to be a positive effect on all aspects as the level of inorganic P increased. The findings of Summers *et al.* (1959) and Vandepopuliere *et al.* (1961) led to their conclusion that growth or body weight is as good of an indicator of P bioavailability as bone ash. This is in cognate to that found later by Potter *et al.* (1995) who states that the body weight gain and bone ash based bioassays results in similar P bioavailability estimations. However

Huyghebaert *et al.* (1980), as well as Nelson & Walker (1964) and Shastak (2012) concluded that using growth for P bioavailability is not suitable.

2.4.4 Quantitative measurements

2.4.4.1 Retention Studies

Qualitative measurements of bioavailability (bone, growth or blood) provide a relative bioavailability. This information has little value when formulating diets as the actual retention values are required to analyse the animal's performance with regard to the diet consumed (Shastak & Rodehutscord, 2013). Furthermore retention values help in understanding the optimal requirements of phosphorus such that there are minimal amounts of P in the bird's excreta (Leske & Coon, 2002). As the amount of phosphorus utilization varies between different feedstuffs, retention values take into account the non-phytate phosphorus as well as the phytate phosphorus which is available, making it vital for feed formulations (Leske & Coon, 2002).

To obtain a retention value for phosphorus one might measure the total collected excreta or with the help of an indigestible marker, the retention value may be calculated. De Groote & Huyghebaert (1997) investigated the bioavailability of P as influenced by two bio-assays, namely toe ash percent and retention. Their experiment followed a seven-day adaption period, with a four-day balance period and collection of the total excreta thereafter. The conclusion was that there was no change in the relative bioavailability of P between the two bio-assays. Van der Klis and Versteegh (1996) and Shastak *et al.* (2012) did similar studies with similar findings.

2.4.4.2 Digestibility

Pre-caecal digestibility is a well-known method used to assess protein quality. This method ensures the post-ileal microbial activity does not affect the results. The importance of this is that in P availability studies, it implies that the urine contribution can be excluded and the urinary excretion of P is a major pathway when intake is higher than required (Rodehutscord, 2009). Therefore the results are not confounded by urinary excretion even if the intake of P is in excess of what is required by the bird (Li *et al.*, 2016). A further advantage is that the sensitivity of the method to the dietary level of P is less than other methods making it a further desired method of P bioavailability assessment (Rodehutscord *et al.*, 2012). This method

provides one with the amount of available P which can be used as a quantitative estimate of phosphorus bioavailability (Li *et al.*, 2016). However, Rodehutscord *et al.* (2012) found the length of the ileum used when collecting the digesta influenced the results.

2.4.5 *In Vitro* Tests

The use of a chick assay to assess availability of P is labour intensive, time consuming and expensive (Waldroup, 1999), and in an attempt to develop a faster and less expensive method, the *in vitro* method was developed. However, this method was only applicable to iP sources and furthermore, the studies conducted have had conflicting results leading to questions being asked regarding the viability of the method (Waldroup, 1999).

An attempt by Bird *et al.* (1945), to link availability of P from different sources using bioassays with chicks to the solubility of P in dilute acid (0.25% HCL) led to the conclusion that solubility is a fast, yet imprecise, method of estimating availability. Gillis *et al.* (1948) further tested the same data in 0.4% HCL and reported that its usefulness in estimating the availability is limited. Gillis *et al.* (1962) later compared the solubility of different calcium phosphate sources to the bone ash percentage in chicks and turkeys; the authors found no correlation between the solubility tests and the bone ash responses. A more recent study by Gueguen (1999) stated that water solubility should not be considered as an acceptable indicator for availability of phosphates. It is therefore clear that no reliable method of evaluation of P availability through *in vitro* is available (Shastak & Rodehutscord, 2013).

2.5 Phosphorus Sources

Phosphorus typically found in broiler diets is said to originate from three sources: plant feed sources, animal feed sources and inorganic minerals (Mc Donald *et al.*, 1997). With plant and animal P being the largest supplier to the diet's total phosphorus content (Viljoen, 2001a). Nonetheless, the amount of P available within these sources is low, making supplementation with inorganic P sources necessary (Soares, 1995; Viljoen, 2001a)

2.5.1 Plant feed phosphorus

It is a well-known fact that the majority of a broiler diet comprises of cereals, grains, seeds and some by-products, all of which contain a certain percentage of phosphorus (0.9-14.2%) (Van der Klis & Versteegh, 1999). However, near 70% percent of this phosphorus is unavailable to

monogastric animals (Viljoen, 2001a) as a particular portion of P is bound in the form of phytic acid and can only be liberated by the enzyme phytase, which is produced during germination (Viljoen, 2001a). Ruminants use endogenous phytase, which is present in the rumen to hydrolyse the phytate P in order to render the phosphorus available (Raun *et al.*, 1956). However, monogastric animals lack this endogenous phytase leaving much of the phytate P unavailable to the animal (Touchburn *et al.*, 1999).

It was later assumed that the amount of P available to monogastric animals is one third of the total P content. On the other hand, Van der Klis & Versteegh (1999) found there to be huge variation in the phytate P of different plant feeds as well as within the sources themselves. Reasons for this may be due to variations between the grains and seeds in terms of the phytase present during germination.

2.5.2 Animal feed phosphorus

There are a number of ingredients commonly used in broiler feeds that are of animal origin and are known to have a high phosphorus content (Viljoen, 2001a). The availability of this P is considerably lower than that of the inorganic P sources, however it has a greater availability than that of the plant phosphorus sources (Soares, 1995). Table 2.3 depicts the phosphorus availability of commonly used animal phosphorus sources used within the broiler industry. The available P ranges from 59% to 74% of total phosphorus for animal feed sources (Van der Klis & Versteegh, 1999). Both Waldroup (1999) and Van der Klis & Versteegh (1999) found a large amount of variation between the sources, indicating that further research is needed to back up these values.

Table 2.3 Phosphorus availability of some animal feed sources measured in broilers (Van der Klis & Versteegh, 1999)

Source	Total P (g/kg)	Available P (% of total P)
Bone meal	76	59
Fish meal	22	74
Meat meal	29	65
Meat and bone meal	60	66

2.5.3 Inorganic feed phosphates

Before the late 1940's, bone meal and phosphate rock were the most utilized P sources in animal feeds. Since then there has been an increased demand for supplements with higher P levels (Viljoen, 2001). The most common of these, which have been produced, tested and used, have been listed in Table 2.4 together with their calcium and phosphorus percentages, respectively. The most common compounds used within South Africa for animal use are mono-dicalcium phosphate (MDCP) and dicalcium phosphate (DCP) (Viljoen, 2001).

Table 2.4 Inorganic feed phosphate supplements being utilized (Viljoen, 2001).

Product	**Ca (%)**	**P (%)**
Monosodium phosphate	0	25
Monocalcium phosphate***	16	22
Monocalcium phosphate**	17	25
Mono-dicalcium phosphate*	18	21
Dicalcium phosphate****	20	18
Dicalcium phosphate**	28	20
Tricalcium phosphate**	39	20
Defluorinated rock phosphate	34	18.5

*= hydrate **= anhydrate ***monohydrate ****= dihydrate

These calcium phosphate sources (MCP, DCP, TCP and MDCP) are mixtures of mono-, di- and tricalcium phosphates formed by reacting phosphoric acid with calcium salts. The ratios of these phosphates, which ultimately leads to the name by which the product is called, depend on the conditions under which the reaction takes place; namely heat, water and pressure. Furthermore, these conditions have an effect on the bioavailability of the product. An example is dicalcium phosphate (DCP) produced through a reaction between defluorinated phosphoric acid and a single or multiple lime source(s). This reaction, depending on conditions during manufacture, results in either an anhydrate or a dehydrate product. These are both DCP, however, the anhydrous form has been found to have an exceptionally lower bioavailability than that of the hydrous form (Viljoen, 2001). Another form of phosphates, known as defluorinated phosphates, are a product of a reaction between phosphoric acid and sodium bicarbonate which is then calcined at 1.25 °C (Waldroup, 1999). This process is seen to be

more difficult to control that that used for calcium phosphate production, and as a result, there is a much greater variability between the biological values compared to the calcium phosphates.

The choice of a particular inorganic supplement over another depends on its bioavailability, cost, impurities it may contain, its' handling properties and accessibility to the local market. Most importantly is the relation between the amount of P it contains and the availability thereof. Table 2.5 is a list of commonly utilized inorganic phosphate sources together with their phosphorus compositions.

Table 2.5 Common inorganic phosphate sources and their phosphorus compositions (Van der Klis & Versteegh, 1999).

Product	**Total P (g/kg)**	**Average Total P (g/kg)**	**Available P (g/kg)**
Monosodium phosphate	224	224	206.1
Monocalcium phosphate	220-228	226	189.8
Mono-dicalcium phosphate*	205-225	213	168.3
Dicalcium phosphate**	175-215	197	108.4
Dicalcium phosphate*	170-210	181	139.4
Defluorinated phosphate	175-195	180	106.2

*hydrous **anhydrous

2.6 Factors Influencing P Availability

There are a large number of factors affecting P availability that include the P form, Ca diet concentration, ratio of Ca: P within the diet, Vitamin D_3, protein, energy, fat, interactions of P with other nutrients, particle size and feed processing, feed consumption, sex, age and growth rate and management factors such as lighting and ambient temperature (Li *et al.*, 2016). Prior to discussing these factors, an understanding of the metabolism of phosphorus is required (Figure 2.2).

Homeostasis of phosphorus is closely related to the metabolism of calcium (Ca) and vitamin D. The skeleton is the primary storage facility for both Ca and P. This is where anionic and cationic forms of P and Ca respectively bind, forming hydroxyapatite, giving the bone matrix its rigidity (Van der Klis & Versteegh, 1999). This bone matrix is continually utilized and reabsorbed. Therefore, coordination of the metabolism of these essential minerals is of utmost

importance to guarantee that their biological demands are met (Li *et al.*, 2016). Van der Klis & Versteegh (1999) state that the relative amounts of both Ca and P controls their utilization. However, the relative amounts available for their metabolic duties are affected by inefficiencies of basic body functions, namely; glomerular filtration, intestinal absorption, renal tubular reabsorption, transfer rates of blood to bone and endogenous losses (Li *et al.*, 2016). These processes are mediated by a number of hormones, the main being the parathyroid hormone and the hormonal form of vitamin D_3 (Klasing, 1998). It has been reported that the control of P metabolism within the gut is low, and high in the kidney, contrary to that of Ca. Furthermore, findings of dietary carbohydrate and protein affecting Ca and P intestinal metabolism are ever increasing (Vitti & Kebreab, 2010). Therefore, factors that regulate intestinal absorption and kidney excretion, together with the kidneys' endocrine regulation of absorption and reabsorption, assist the body's P homeostasis. With reference to Figure 2.2, it is concluded that the intestine, bone and kidney are integral to Ca and P homeostasis. A reduced concentration of Ca results in parathyroid hormone (PTH) secretion by the parathyroid glands. Parathyroid hormone then stimulates increases in bone resorption and Ca reabsorption and renal excretion of P. The release of PTH also stimulates formation of vitamin D_3 within the kidneys. This increases absorption of Ca and P within the intestine, although P absorption is notably lower than that of Ca. It is these raised blood Ca levels, which creates a negative feedback loop maintaining homeostasis (Li *et al.*, 2016).

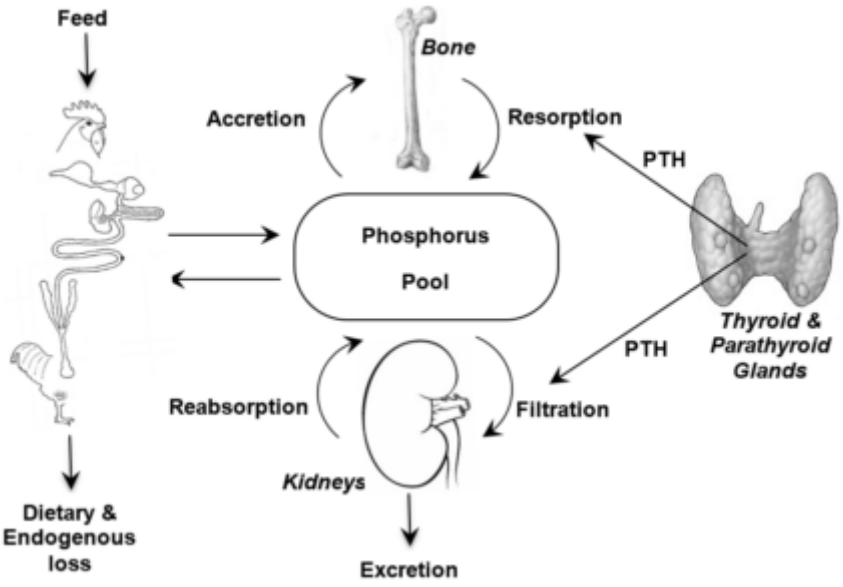

Figure 2.2 Phosphorus metabolism (Li *et al.*, 2016)

2.6.1 Calcium and Ca: P Ratio

Calcium is essential to bone and shell formation as well as blood clotting and muscle contraction (Li *et al.*, 2016). The bioavailability of calcium within raw plant materials is relatively low as a result of the high phytate content (Li *et al.*, 2016). An increase in the concentration of Ca and P within the diet can affect the digestion of both these minerals. Too high levels of Ca and low levels of P has been shown to have negative effects on performance in poultry (Li *et al.*, 2016). The elevated Ca concentration causes an increase in the pH of the gastrointestinal tract which is turn causes a decrease in P absorption and retention (Hurwitz & Bar, 1965; Li *et al.*, 2016). The increased Ca concentration also increases the gastrointestinal pH and decreases phytate hydrolysis through the increased binding of Ca to phytate phosphorus (Guinotte *et al.*, 1995; Manangi & Coon, 2008). High levels of P also decreases the absorption of Ca from the gut (Keshavarz & Austic, 1990).

Due to the effect dietary Ca levels has on P availability, deliberation has been held with regards to maintaining a constant level of Ca or a Ca: P ratio when performing P availability tests. Nelson & Walker (1964) found that a Ca: P ratio of 2: 1 is ideal, as it closely resembles that retained by the chickens and meets their requirements. This was later confirmed by Leske & Coon (2002) and Manangi & Coon (2008) who both found this ratio to maximize retention of P.

2.6.2 Vitamin D

As mentioned in section 2.4.1, vitamin D plays has an integral role in Ca and P metabolism. It is for this reason that diets now include vitamin D as a supplement. Vitamin D's role in absorption of Ca and P and regulation of the secretion of PTH increases bone ash density which is linked to a decreased occurrence of bone injuries and disorders (Driver *et al.*, 2005; Garcia *et al.*, 2013). The use of vitamin D supplements provides metabolised vitamin D to the animals, causing an increased efficiency and reduced energy use (Garcia *et al.*, 2013). Studies have also shown that the form of vitamin D may play a part in reduced P excretion (Garcia *et al.*, 2013). Isomers of vitamin D also have the potential to act together with microbial phytase, causing an improved P utilization in chicken diets (Roberson & Edwards, 1994; Mitchell & Edwards, 1996). Edwards (1993) reported that new isomers of Vitamin D have the ability to enhance the intestinal phytase within broiler diets. Han *et al.* (2012) found the use of one alpha-hydroxycholecalciferol (1α-OH D_3) to improve chicken growth, meat colour as well as the tibia

quality when birds were fed P-deficient diets, further illustrating the importance and efficiency of vitamin D in P retention. However, excess addition of vitamin D have shown to have no further effect on productivity after saturation levels within the body are met (Li *et al.*, 2016) and much like many of the other minerals, knowledge on its role in P absorption on a molecular level, is still limited.

2.6.3 Interactions

Minerals are crucial to everyday life due to their roles as protein stabilizers, enzyme cofactors, regulators of acid-base balance and the secondary messengers (Hemati Matin *et al.*, 2013). Previously, the study of minerals and their requirements focused on one individual mineral at a time. This however has been proven incorrect as interactions between minerals have been reported (Nugara & Edwards, 1963; Davies & Reid, 1979; Henry & Miles, 2000). Now, animal nutritionists agree that there is some degree of interaction between all dietary nutrients (Henry & Miles, 2000), and there is a general understanding that to obtain the desired response from an animal, there are ideal concentrations of each nutrient in relation to others (Hemati Matin *et al.*, 2013). Nonetheless, we do not have a complete understanding of the impact of these interactions on the absorption, excretion, storage and utilization of other minerals (Henry & Miles, 2000; Hemati Matin *et al.*, 2013). The known interactions P has with other minerals are depicted in Figure 2.3. The most prominent of these interactions is that with Ca and magnesium (Mg)(Hemati Matin *et al.*, 2013; Kleyn, 2013). The importance of P with Ca has been highlighted in sections 2.6.1. Phosphorus can be found all over in nature; however, it is never available in its free state. It is known to combine spontaneously and vigorously with oxygen (Hemati Matin *et al.*, 2013). Excess levels of Ca and P have been shown to cause an increase in chickens' Mg requirements (Nugara & Edwards, 1963). An increase in Mg by 0.2-0.4% has shown to alleviate these effects (Chicco *et al.*, 1967). However, an increase in Mg by 0.6% negatively affected bone growth and mineralization irrespective of the dietary Ca and P levels. This suggests that Mg metabolism may be related to the hormones and enzymes responsible for bone mineralization (Hemati Matin *et al.*, 2013).

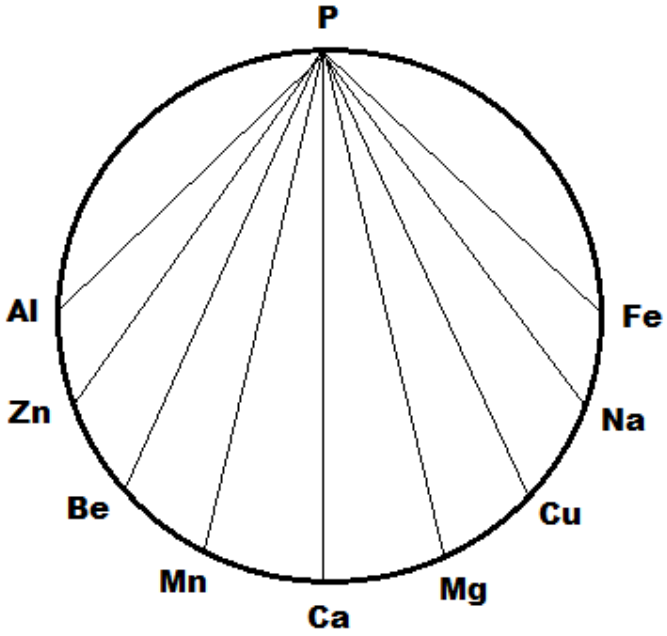

Figure 2.3 Some of the known interactions between phosphorus and other minerals in the diet (adapted from Kleyn, 2013).

2.6.4 Phytate

Phytate, a salt of phytic acid, is a compound which is naturally found within plant feedstuffs (Reddy *et al.*, 1982). Phosphorus and inositol are primarily stored in the form of phytate within seeds (Hídvégi & Lásztity, 2002). Phytate also plays a role in maintaining homeostasis of P levels and is very important for germination of seeds as well as plant growth itself (Lott *et al.*, 2000). Phytic acid has been found to form insoluble salts by creating complexes with divalent or trivalent cations rendering their availability to absorption within the digestive system low (Cheryan & Rackis, 1980; Singh, 2008). Phytic acid is known to reduce the availability of Ca (Lönnerdal *et al.*, 1989), P (Harrold *et al.*, 1983), zinc (Davies & Olpin, 1979; Lönnerdal *et al.*, 1989), Mg (Brink *et al.*, 1991) and iron (Brune *et al.*, 1992).

Phytate bound phosphorus (in the form of phytic acid) originates from plant feed stuffs and a large portion of this is unavailable to monogastric animals (Viljoen, 2001). Therefore, there is need for supplementation of P using iP. Presence of this phytate bound P within the basal diet may cause reduced P availability from a highly available source (Harrold *et al.*, 1983). However, there is reason to believe that excess available P within the basal diet could cause

further reduced P absorption from the test source as well (Viljoen, 2001; Leske & Coon, 2002; Li *et al.*, 2016). It is therefore clear that the P composition of the diet as well as any factors that may affect phytate phosphorus use within the diet may cause misinterpretations of the results.

2.6.5 Phytase

Phytase is a digestive enzyme which catalyses the release of P from phytic acid, and is currently the only enzyme known to break this bond (Shaw *et al.*, 2010). In essence, the inositol phosphate molecule is liberated to revert back to inositol, freeing the phosphate molecule, giving it the potential to be absorbed and utilized by the animal (Viljoen, 2001). However, this enzyme is found to be deficient within monogastric animals, therefore phytate hydrolysis is low (Applegate *et al.*, 2003; Classen *et al.*, 2010). A large portion of P in cereal grains used in poultry feed is bound within phytic acid and this is otherwise known as phytate phosphorus (Viljoen, 2001). This P has varying availability but much of it is unavailable (Viljoen, 2001; Leske & Coon, 2002). The low P availability as well as high levels of phytate phosphorus loss through excretion causes for dietary supplementation with inorganic P. Loss of this phytate phosphorus is detrimental; firstly, P is a big role player in much of the functions within the body as mentioned in section 2.2.2 and the P in the manure can have environmental implications in the form of for example, water eutrophication (Waldroup, 1999; Driver, 2004; Rodehutscord, 2009; Classen *et al.*, 2010; Shastak, 2012; Li *et al.*, 2016).

Supplementation with exogenous phytase significantly increases phytate phosphorus utilization in poultry (Baxter *et al.*, 2003; Angel *et al.*, 2006; Bougouin *et al.*, 2014). Not only does it hydrolyse the bond between phytate and P but also the bonds between phytate and other minerals (mentioned in section 2.6.3) leading to an overall increase in the availability of these minerals (Kornegay *et al.*, 1996). However, the phytate and dietary phosphorus levels have the potential to influence phytase efficiency. Diets with varying phytic acid and low npP levels were supplemented with exogenous phytase (Ravindran *et al.*, 2000). It was reported by the authors that an increase of 18.6% in phytate phosphorus digestibility was found between diets with phytic acid levels of 10.4 and 15.7 g/kg. Both diets had a low npP level of 0.23 g/kg. However, the phosphorus digestibility decreased when the diets had adequate npP levels.

Bearing in mind that phytase liberates other minerals; it is possible that this hydrolysis of the bonds between phytate and phytate-bound proteins increases the availability of the proteins.

Sebastial *et al.* (1997) reported that phytase would reduce the phytate levels within a diet and consequently improve amino acid digestibility through reducing the effects phytate has on proteases. Moreover, due to the expense of phytase supplement in diets, the enhanced protein utilization improves the cost effectiveness of the phytases.

2.6.6 Hydration state

The manufacturing of iP results in the formation of either a hydrous or an anhydrous product. This is subject to the quality of raw materials used and the conditions during manufacturing (Viljoen, 2001). The hydrous form has been found to have a much greater P availability compared to the anhydrous form (Potter *et al.*, 1995; De Groote & Huyghebaert, 1997; Van der Klis & Versteegh, 1999; Viljoen, 2001). Potter *et al.* (1995) reported monohydrated MCP to have the highest availability than any of the other sources. De Groote & Huyghebaert (1997) revealed the availability values for a hydrous and anhydrous DCP to be 74.2% and 63.3%, respectively and Van der Klis & Versteegh (1999) reported values of 77 and 55%, respectively for the same sources.

2.6.7 Inorganic phosphate source

It is now common knowledge to assume that not all phosphorus found within the diet is 100% available. However, inorganic sources have been identified to contain the highest P availability and that differences are present between the various sources (Viljoen, 2001). In addition, as mentioned earlier, a portion of plant phosphates are bound to phytic acid causing it to be unavailable to chickens hence its mention being unnecessary.

A test using the three calcium phosphates (mono-, di- and tri-) performed by Gillis *et al.* (1962), found the monocalcium phosphate (MCP) to have the highest P availability, followed by dicalcium (DCP) with tricalcium phosphate (TCP) having the lowest P availability. However, there have been variations in the actual values of P bioavailability. Findings by Potchanakorn & Potter (1987) reported average values of P bioavailability of 92.6, 81.2 and 69.6% for MCP, DCP and defluorinated phosphate, respectively. This is similar to findings of Gillis *et al.* (1962) in terms of MCP and DCP P availability however, exact value differences between the two studies are evident. In one trial by De Groote & Huyghebaert (1997), values of 78.1 and 74.2% for MCP and DCP respectively where reported whilst in the second trial the values increased by 8.7 and 9.8%, respectively. However, defluorinated rock phosphates were not tested in the

second study. This illustrates how availabilities can differ between sources as well as within the sources. Table 2.6 shows the variation in available P between the inorganic phosphates to be between 55 and 92%. An earlier study by Huyghebaert *et al.* (1980) tested a number of commercial phosphates for broilers against a reference source (di-Na-phosphate). Particularly two defluorinated rock phosphates were evaluated; percentage of P were 18.5 and 18.11 with available P at 96 and 94%, respectively. A later study by Potchanakorn & Potter (1987) found similar phosphorus percentage results when testing two defluorinated rock phosphates. However, the reported availabilities of the two sources were substantially lower at 69.9 and 75.5%, respectively.

Table 2.6 Common inorganic feed phosphates and their availabilities (Van der Klis & Versteegh, 1999)

Phosphate Source	Available P (% of total P)	Available P (g/kg)
Dicalcium Phosphate (anhydrous)	55	108.4
Dicalcium Phosphate (hydrous)	77	139.4
Monocalcium Phosphate	84	189.8
Mono-dicalcium Phosphate	79	168.3
Monosodium phosphate	92	206.1

2.7 Conclusion

In light of all that has been discussed above, the importance of phosphorus in the broiler industry is evident. More specifically, the importance of formulating a diet to meet the needs of a broiler without over supplying P, and in doing so, removing the implications that excess P has on the environment and also increasing the farmer's profit margins is self-evident. However, all sources of phosphorus vary in terms of availability and a number of effectors within the diet may alter these values further. Therefore, it is of utmost importance to ensure the availability of the P in the source is known, making sure the method used to assess availability allows for accurate assignment of a P bioavailability value before using it in production. Furthermore, the method needs to be repeatable and be applicable to any phosphorus source.

Phosphoric acid (PA) in poultry diets is not widely used due to no viable PAs being available. However, a defluorinated PA and a defluorinated and desulfonated PA has become available locally. Therefore, it is important to conduct a study assessing the bioavailability of P in the PAs and furthermore determine what effects the PAs have on production parameters and physical carcass characteristics whilst being subjected to the same conditions.

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Chapter 3

The evaluation of phosphorus bioavailability and total intestinal tract digestibility of phosphoric acid in broiler diets

3.1 Abstract

This study assessed the bioavailability of phosphorus (P) in defluorinated phosphoric acid, as well as the nutrient (fibre, fat, ash and protein) and mineral digestibility and apparent metabolisable energy of broiler chicken diets supplemented with defluorinated PA. Fifty day-old chicks were assigned to one of five dietary treatments. A summit and a dilution diet were mixed to contain the same amount of available P. The summit diet used a standard mono-dicalcium phosphate (MDCP) as its source of P supplement and the dilution diet used defluorinated PA as its source of P supplement. The treatment diets were then mixed to contain different ratios of the summit and dilution diet. These ratios were 100:0, 75:25, 50:50, 25:75 and 0:100 respectively. From day 0-14 the birds were on the same diet. The birds were then placed into individual cages that had been allocated with one of the five treatment diets. The birds were allowed to adapt to their respective feeds for four days. Thereafter, the trial commenced, where the faecal samples, daily intake and refusal samples were collected every morning. Treatment significantly affected the P bioavailability, as well as the coefficient of the total tract digestibility (CTTD) values of P, sodium (Na), iron (Fe), copper (Cu), zinc (Zn), manganese (Mn) and aluminium (Al). The P bioavailability was the highest for the diet with 100% PA as its source of P supplement. This diet further revealed ideal CTTD values for all nutrients and minerals.

3.2 Introduction

The level of nutrient ingestion, digestion and absorption can be related to the performance level of an animal. Animals with access to highly digestible feed tend to perform better than those fed feed with poor digestibility. Superior growth performance has been found in broilers fed on feeds with particularly high dry matter, ether extract, crude protein and phosphorus digestibility values (Emami *et al.*, 2013; Thiamhirunsopit *et al.*, 2014). Formulation of modern broiler diets requires ingredients that have a known digestibility value, which can be incorporated into the formulation, allowing for the best performance from the animal and

reduced waste in terms of undigested nutrients. Chemical analysis can be used to determine the value of an ingredient for supplying a particular nutrient. However, actual values can only be obtained after allowing for losses, which occur during digestion, absorption and metabolism (Borin *et al.*, 2002).

Phosphorus (P) is the second most abundant element for a broiler and plays a vital role in both the soft and hard tissue of the body (de Carvalho Mello *et al.*, 2012). The requirements and absorption of P are influenced by numerous factors (De Groote & Huyghebaert, 1997; Kornegay, 2000; Driver, 2004; Angel, 2010; de Carvalho Mello *et al.*, 2012; Shastak, 2012; Li *et al.*, 2016). Broiler diets consist primarily of plant origin, which has variable levels of total P (tP)(0.9-17.2%)(Van der Klis & Versteegh, 1999). However, a large portion of this P (70%) is bound to phytate and monogastric animals lack phytase enzymes, which liberates P from its chemical bond to phytate (Classen *et al.*, 2010); therefore, much of the phytate bound P is considered unavailable (Viljoen, 2001a; Leske & Coon, 2002). This has led to broiler diet supplementation with inorganic phosphates (iP) with greater levels of available P (aP)(Viljoen, 2001a). However, P supplements are expensive and P excretion caused by an oversupply of P is known to be damaging to the environment (De Groote & Huyghebaert, 1997). Diets therefore need an accurate supply of P to alleviate these issues as far as possible and knowledge of bioavailability values of the iP sources (Rodehutscord, 2013) as well as the requirements during different production stages is essential (Viljoen, 2001b).

The full absorption and utilization of an element is never attainable, as losses occur through normal digestion and metabolism. Many techniques have been developed to determine what portion of the element an animal will utilize and this has led to a number of terms being used, which can cause confusion. The most often used terminology is digestibility and bioavailability and these are frequently confused with one another (Viljoen, 2001b). Using P as an example, “digestibility is the amount of phosphorus ingested minus the amount voided in the faeces, including exogenous losses” whilst bioavailability is “the portion of a mineral that is retained in the body” (Viljoen, 2001b).

Studies on P bioavailability date back as far as the late 1920’s. Thereafter, determination and comparison of the available P content in different P sources have developed considerably, with a number of published studies testing the P availability of commercial and experimental phosphate sources within the broiler industry (Gillis *et al.*, 1962; Nelson & Walker, 1964;

Huyghebaert *et al.*, 1980; Potchanakron & Potter, 1987; Potter *et al.*, 1995; Lima *et al.*, 1997; De Groote & Huyghebaert, 1997; Van der Klis & Versteegh, 1999; Rama Rao & Ramasubba Reddy, 2001a; Leske & Coon, 2002; Li *et al.*, 2016)

Factors that influence the choice of P supplement include the bioavailability, cost, accessibility, handling properties and the chemical composition (Payne, 2005). Within South Africa, mono-dicalcium phosphate (MDCP) and dicalcium phosphate (DCP) are the commonly used iP sources (Viljoen, 2001a) that have 79%, and 77% available P respectively (Van der Klis & Versteegh, 1999). All of these products are made by reacting calcium salts with phosphoric acid (PA)(Lima *et al.*, 1997; Viljoen, 2001a). However, the iP products have been known to contain undesirable elements owing to the nature of the raw materials used. These elements are fluorine (F), lead (Pb), mercury (Hg) and arsenic (As), with the possibility of being toxic to animals and negatively influencing P availability (Viljoen, 2001a; Rama Rao & Ramasubba Reddy, 2003) In light of this, the objectives of this study are:

i. To investigate the bioavailability of defluorinated phosphoric acid by means of a digestibility study.
ii. To evaluate the effects of defluorinated phosphoric acid on nutrient and mineral apparent digestibility coefficients.

3.3 Materials and Methods

3.3.1 Birds and housing

Before any trials were performed, ethical clearance was obtained from Stellenbosch University Ethics Board, clearance number SU-ACUM13-00006. For the purpose of this trial, 50 day-old Cobb 500 chicks were obtained from a commercial hatchery and transported to Stellenbosch University experimental farm, Mariendahl, where the trial was conducted. Five cages (0.9m by 0.6m) were initially used to house the chicks for the first 14 days at a stocking density of 10 chicks per cage. Thereafter, the birds were separated into individual cages (0.45m by 0.6m), which had been allocated with one of five dietary treatments. Each cage was equipped with a nipple drinker and feeder. After an adaption period and the sample collection period, the birds were returned to their initial cages, with the same stocking density as before being separated, and grown out until slaughter at 35 days of age. The lighting and environmental humidity and temperature within the house were maintained according to the Cobb 500 standards throughout

the trial (Table 3.1 and 3.2, respectively). Water was supplied *ad libitum* and ventilation was set at a minimum of six air changes per hour.

Table 3.1 Cobb 500 standard lighting program for broilers fed to a slaughter weight of <2.5kg.

Age in days	Number of dark hours	Change in lighting hours
0	0	0
1	1	1
100-160 grams	6	5
Five days before slaughter	5	1
Four days before slaughter	4	1
Three days before slaughter	3	1
Two days before slaughter	2	1
One days before slaughter	1	1

Table 3.2 Cobb 500 environmental humidity and temperature standard for broilers reared to 42 days of age.

Age in days	Relative humidity	Temperature
0	30-50	34
7	40-60	31
14	40-60	27
21	40-60	24
28	50-70	21
35	50-70	19
42	50-70	18

3.3.2 Treatments, diets and trial procedure

Treatments were according to diet. For the first 14 days, only a standard starter control diet was supplied, using commercially available mono-dicalcium phosphate (MDCP) as the

supplemented phosphorus source. Formulation was performed using Winfeed. Thereafter, two grower and finisher diets were formulated, namely a summit diet, containing a standard MDCP as the supplemented phosphorus source, and a dilution diet containing a defluorinated phosphoric acid as the supplemented phosphorus source. Table 3.3 shows the composition of the diets. The dilution and summit diets were blended in ratios of 100:0; 75:25; 50:50; 25:75 and 0:100 respectively. These diets were then used for the remaining duration of the trial. All diets were mixed at Mariendahl experimental farm and fed as mash diets.

At day 14, birds were separated into individual cages where they adapted to their respective experimental diets for four days. From day 18, to the end of the 4 day sampling period, feed intake and refusal were measured to determine daily feed intake. On day 18, the cages were fitted with faecal collection trays, which had been outlined with a clear sheet of plastic. Faecal trays were cleared daily. Each bird was supplied with a specific amount of feed, in grams, based on the amounts obtained during the adaptation period. These amounts were bird specific.

Table 3.3 Ingredient (%) and calculated nutrient composition of the broiler grower and finisher diets used in the trial

Ingredients	Grower		Finisher	
	Dilution	**Summit**	**Dilution**	**Summit**
Maize	51.5	51.1	57.6	57.2
Soybean full fat	15.0	15.0	15.0	15.0
Soybean 46	24.8	24.9	18.8	18.9
L-lysine HCl	0.2	0.2	0.2	0.2
DL methionine	0.4	0.4	0.3	0.3
L-threonine	0.1	0.1	0.1	0.1
Vit+min premix*	0.2	0.2	0.2	0.2
Limestone	1.5	2.2	1.5	2.1
Salt	0.2	0.2	0.2	0.2
MDCP	1.5		1.3	
Sodium bicarbonate	0.18	0.2	0.2	0.2
Phosphoric acid		0.9		0.9
Oil - sunflower	4.5	4.6	4.6	4.7
Calculated nutrient composition				
AME (MJ/kg)	13.2	13.2	13.4	13.4
Dry matter (%)	88.9	88.9	88.7	88.7
Crude protein (%)	21.9	21.9	19.6	19.6
Crude fibre (%)	3.2	3.2	3.0	3.0
Crude fat (%)	9.5	9.6	9.8	9.9
Calcium (%)	0.9	0.9	0.9	0.9
Phosphorous (%)	0.7	0.7	0.6	0.7
Available phosphorous (%)	0.5	0.5	0.4	0.4
Lysine (%)	1.4	1.4	1.2	1.2
Methionine (%)	0.7	0.7	0.6	0.6
Threonine (%)	0.9	0.9	0.8	0.8

*Vitamin and mineral premix.
AME- apparent metabolisable energy.
MDCP- mono-dicalcium phosphate known as Kynofos 21, a South African produced product with a known ratio of monocalcium phosphate to di-calcium phosphate ratio of 75:25.

3.3.3 Data collection

The weight of each bird was recorded at the beginning (day 14) and end (day 22) of the digestibility trial. Samples of each treatment fed were randomly collected, sealed and stored for further analysis. Feed intake and refusal were measured daily and random samples of the refusal, per cage, were collected. Every morning on days 19 to 22, the dropped excreta were collected at 09:00. The faeces collections were cleaned of any visible foreign objects to faecal matter, and frozen at -18^{o}C until further analysis.

3.3.4 Feed and faecal analysis

All analytical procedures were performed at Stellenbosch University, in the Department of Animal Science. However, mineral analysis was performed at the Western Cape Department of Agriculture, Elsenburg.

3.3.4.1 Dry matter and ash determination

Dry matter (DM) and ash were determined according to the Association of Official Analytical Chemists International (AOAC, 2002 & 2006), official method 934.01 and 942.05, respectively. A 2g sample was weighed into a porcelain crucible, with a predetermined weight, and placed into an oven at 100°C for 24 h. The weight of the sample and crucible after 24 h was then used to calculate dry matter percentage using equation 3.1. Thereafter, these crucibles were placed in a furnace at 500°C for 6 hours to ash and this weight was subsequently used to calculate ash percentage using equation 3.2. All samples were analysed in duplicate.

Equation 3.1

$$\text{Moisture \%} = \frac{(A+B) - C}{B} \times 100$$

DM % = 100 - Moisture %

Where:

A = weight of empty crucible

B = weight of air dried sample

C = weight of crucible and dry sample.

Equation 3.2

$$\text{Ash \%} = \frac{\text{D - A}}{\text{sample mass}} \times 100$$

Where:

A = weight of empty and dry crucible

D = weight of crucible and ash

Thereafter the organic matter was calculated as follows (Equation 3.3):

Equation 3.3

Organic matter % = 100 - Ash %

3.3.4.2 Crude fat determination

The Diethyl Ether Reagent extraction method as described by the AOAC (2006), official method 920.39 was used for crude fat determination method. This method incorporates boiling a 2 g sample with added ethanol and hydrochloric acid 38% for 30 minutes. The sample was then placed into a separating funnel with 25 ml diethyl ether added and shaken for 1 minute. This was then repeated, however, using petroleum ether and allowed to settle until a distinctive division could be seen where by the top layer was poured out into a fat cup of a pre-determined weight. This was then repeated with 15 ml of diethyl ether and petroleum ether. The fat cups were then placed on a 60 °C sand bath to allow all the ether to evaporate for ±30 minutes. The weight thereafter was used to calculate fat percentage.

3.3.4.3 Crude protein determination

Crude protein content was determined through measuring the total nitrogen content with the use of a LECO FP528 machine, as described by the AOAC (2002), official method 992.15. The LECO FP528 was first calibrated with ALFALFA (LECO reference material), which has a known nitrogen content of 3.62 ± 0.5. An aluminium foil crucible with 0.1g of feed or faeces

was then placed into the LECO FP528. The crude protein of the sample was then calculated using equation 3.4.

Equation 3.4

Crude protein (%) = Nitrogen (%) × 6.25

3.3.4.4 Crude fibre determination

Crude fibre analysis was performed with the ANKOM Fibre analyser using the Filter Bag Technique. Approximately 1 g of sample was sealed, through heat, in a weighed ANKOM filter bag. These where soaked in petroleum ether for 10 minutes, to de-fat the sample, and then removed and allowed to air dry. The air-dry bags were then placed into the ANKOM and agitated at 100 ºC with approximately 2 litres of sulphuric acid solution (0.255N) for 40 minutes. After the 40 minutes, the H_2SO_4 solution was removed and hot water used to rinse the samples twice whilst still within the ANKOM. The samples were then agitated in a similar manner as before however, using a sodium hydroxide solution (0.313N). Subsequent rinsing took place, twice, after the 40 minutes of agitation. Thereafter, the samples were removed and soaked in acetone for 5 minutes and allowed to air dry. After air-drying, the samples were placed in a 100 ºC oven for two to four hours. The samples were then removed and the weights recorded before ashing at 500 ºC for five hours. The ash samples where then weighed and the crude fibre determination made using equation 3.5.

Equation 3.5

$$\text{Crude fibre } \% = 100 \times \frac{W_3 - (W_1 \times C_1)}{W_2}$$

Where:

W_1 = bag tare weight

W_2 = sample weight

W_3 = weight of organic matter (loss of weight on ignition of bag and fibre)

C_1 = Ash corrected blank bag factor (loss of weight on ignition of blank bag/original blank bag).

3.3.4.5 Gross energy

The gross energy values were determined using the IKA calorimetric system C200. After calibration by combustion of benzoic tables with known calorific value, samples were pelleted and placed individually into the decomposition vessel. The vessel was then filled with oxygen to a pressure of 3000 kPa and placed into the calorimeter for combustion. The MJ/kg reading was taken as the gross energy value.

3.3.4.6 Bioavailability

Calculation of bioavailability of P was calculated according to equation 3.6. This calculation takes into account the P that not only originates from the iP but also from the feed components (maize, soybean full fat and soya 46).

Equation 3.6

$$\text{Bioavailability (\%)} = \frac{\text{Total ingested P - Excreted P}}{\text{Total ingested P}} \times 100$$

Where:

Total ingested P = P originating from maize, soybean full fat, soya 46 and the supplemented P.

3.3.4.7 Coefficient of total tract digestibility

The coefficient of total tract digestibility of each nutrient analysed was calculated using equation 3.7 all on dry matter bases.

Equation 3.7

$$\text{Nutrient consumed} = \text{Nutrient}_{\text{analysed in feed}} \times \text{Dry matter}_{\text{intake}}$$

$$\text{Nutrient excreted} = \text{Nutrient}_{\text{analysed in excreta}} \times \text{Dry matter}_{\text{excreta}}$$

$$\text{Digested nutrient} = \text{Nutrient consumed - Nutrient excreted}$$

$$\text{Coefficient of total tract digestibility} = \frac{\text{Digested nutrient}}{\text{Nutrient consumed}}$$

3.3.4.8 Mineral analysis

Feed and faecal samples were sent to the Western Cape Department of Agriculture located at Elsenburg, for mineral element analysis. Analysis was completed according to the combustion method 6.1.1 described by Agricultural Laboratory association of Southern Africa (ALASA, 2007). Each ashed sample received 5 ml of 6 M hydrochloric acid and was subsequently placed in a 50ºC oven for 30 minutes. Thereafter 35 ml of distilled water was added and filtered into a bottle. Extra distilled water was added to reach a final volume of 50 ml. Elements were measured on an iCAP 6000 Series Inductive Coupled Plasma (ICP) Spectrophotometer (Thermo Electron Corporation, Strada Rivoltana, 20090 Rodana, Milan, Italy) fitted with a vertical quartz torch and Cetac ASX-520 autosampler. The element concentrations were calculated using iTEVA Analyst software. Elements measured were phosphorus, calcium, magnesium, potassium sodium, iron, copper, zinc, manganese, boron and aluminium.

3.4 Statistical analysis

All statistical analysis was performed using the general linear models procedure in STATISTICA (Dell Inc., 2016). The general assumptions of normal and homoscedastic data was first tested before any other analyses. All dietary treatment effects were analysed by one way (ANOVA) analysis of variance at a significance level of 0.05. If the data was significant, Bonferroni's *post hoc* test was performed to analyse the difference between the diets.

3.5 Results and discussion

3.5.1 Bioavailability

The first objective of the current study was to investigate the P bioavailability of defluorinated phosphoric acid. As Table 3.4 shows, there is a significant difference in P bioavailability between the dietary treatments. Treatment 0:100 has a greater ($p \leq 0.05$) P bioavailability than the other treatments and diets 75:25 and 50:50 have the lowest ($p \leq 0.05$) P bioavailability. Treatment 0:100 comprises solely of the summit diet, which has the test P source (defluorinated PA) as its supplementary P source.

Knowledge of a nutrients bioavailability is important for accurate feed formulation (Viljoen, 2001b; Singh, 2008; Rodehutscord *et al.*, 2012; Li *et al.*, 2016), which in turn ensures little and potentially harmful nutrient loss to the environment (Viljoen, 2001b; Leske & Coon, 2002;

Maguire *et al.*, 2005; Rodehutscord, 2009, 2013) and reduces feed costs (Viljoen, 2001b; Rodehutscord *et al.*, 2012; Rodehutscord, 2013). Previously, the available P (aP) of plant feed sources was taken as the total phosphorus (tP) minus the phytate P, because phytate P was assumed completely unavailable and non-phytate phosphorus (npP) was also assumed to be fully digested. However, Van der Klis and Versteegh (1996) found the aP in plant feed sources to be greater than the tP minus phytate P, and npP to be between 55-92% available for use by broilers. Furthermore, inorganic phosphate (iP) sources were considered as npP and therefore are seen to be completely available. This however not the case, highlighting the need to assess the P availability of all feedstuffs (De Groote & Huyghebaert, 1997; Viljoen, 2001b).

Table 3.4 Mean (± standard error) calculated phosphorus bioavailability from chickens fed diets which had different ratios of a dilution diet that had mono-dicalcium phosphate as its phosphorus source and summit diet that had phosphoric acid as its phosphorus source.

Treatment	Bioavailability (%)
100:0	$79.087^{b} \pm 1.506$
75:25	$67.395^{d} \pm 2.206$
50:50	$63.409^{d} \pm 1.992$
25:75	$73.747^{c} \pm 1.272$
0:100	$84.460^{a} \pm 0.746$

[a,b] means within columns that have different superscripts differ significantly ($p \leq 0.05$)
100:0= 100% dilution diet and 0% summit diet.
75:25= 75% dilution diet and 25% summit diet.
50:50= 50% dilution diet and 50% summit diet.
25:75= 25% dilution diet and 75% summit diet.
0:100= 0% dilution diet and 100% summit diet.

Due to the increased realization of P importance in poultry nutrition, numerous studies have assessed the bioavailability of various P sources (Heuser & Norris, 1926; Baird & MacMillan, 1942; Bird *et al.*, 1945; Gillis *et al.*, 1948, 1954, 1962; Summers *et al.*, 1959; Hurwitz, 1964; Nelson & Walker, 1964; Nelson, 1967; Pensack, 1974; Huyghebaert *et al.*, 1980; Potchanakron & Potter, 1987; Van der Klis & Versteegh, 1996; Lima *et al.*, 1997; Leske & Coon, 2002; Shastak, 2012). These studies show variation between their bioavailability results, as well as with the current study. The variation might be attributed to the method of bioavailability determination (see Chapter 2.4). Potchanakorn and Potter (1987) calculated the relative bioavailability of MCP, DCP and defluorinated phosphate firstly using body weight and

secondly using toe ash percentage as the response criterion. For body weight, the P bioavailability values of the MCP, DCP and defluorinated phosphate were 89.7%, 78.9% and 71.5% respectively. However, the use of toe ash percentage resulted in values of 95.4%, 83.7% and 73.7%, respectively for the same sources. Potter *et al.* (1995) also used body weight and toe ash as response criteria for calculating P bioavailability of phosphoric acid (PA) and MCP using DCP as the reference source (set at 100% available). Bioavailability values for the two response criterion (body weight and toe ash) were reported to be 89% and 97%, respectively for PA, and 112.9% and 110.7%, respectively for MCP. These results differ greatly from that of the current study and this may be due to different methods of determination, as a digestibility procedure was used to assess bioavailability in the present study. Van der Klis and Versteegh (1996) reported MDCP to have a 79% available phosphorus by means of a retention study, which is similar to that found in the current study. It is therefore clear that the method of determination plays a role in the results and so it is necessary to determine a standard method which should be used through all bioavailability studies in order to alleviate these differences attributed to methodology. Further cause for differences may be differences in the P sources themselves. As mentioned in section 3.2, MCP and DCP are produced through reacting PA with calcium salts. However, both MCP and DCP are not pure products but mixtures of MCP and DCP (Lima *et al.*, 1997). Therefore, a MCP source must contain at least 80% MCP to be classified as such. Mono-dicalcium phosphate, much like DCP, has a wide variation in bioavailability and composition between two different sources of the same name. For example, a MDCP may have a MCP:DCP ratio of 50:50 to 80:20 possibly giving rise to a large degree of variation (Viljoen, 2001a; b). Furthermore, the production of calcium phosphates can be either hydrated or anhydrated, depending on the manufacturing process, with the hydrous form being of greater bioavailability than the anhydrous form (Viljoen, 2001a).

The levels of P within a supplemented P source can lead to differences in P bioavailability (Payne, 2005). The reference source used in the current study, known as Kynofos 21, is a South African produced MDCP with a known ratio of MCP:DCP of 75:25 (Viljoen, 2001a; KK Animal Nutrition (Pty) Ltd, 2010). When looking at earlier studies on P bioavailability, which have used both MCP and DCP, it is clear that MCP is normally more available to the animal than DCP (Huyghebaert *et al.*, 1980; Potchanakron & Potter, 1987; Potter *et al.*, 1995; Van der Klis & Versteegh, 1996; Viljoen, 2001a). Viljoen (2001a) states that Kynofos 21 has a favourable bioavailability due to its high ratio of MCP:DCP and therefore does not differ

significantly from the bioavailability results of pure MCP's. The defluorinated PA used in the current study has a known P content of 20.9-22.7% and the MDCP has a known P content of 21% (KK Animal Nutrition (Pty) Ltd, 2010). As both sources used in the summit and dilution diet have the same levels of P within their composition, P levels cannot be the cause for the differences in the bioavailability.

Animal nutritionists are now in agreement that there is some degree of interaction between mineral elements within a diet (Henry & Miles, 2000). Although, a full understanding of these interactions and their effects on absorption, storage, utilization and excretion of other minerals is not readily available (Henry & Miles, 2000; Hemati Matin *et al.*, 2013). The interaction of calcium (Ca) and P however, has been extensively studied (Buckner *et al.*, 1930; Vandepopuliere *et al.*, 1961; Nelson & Walker, 1964; Chicco *et al.*, 1967; Harrold *et al.*, 1983; Guinotte *et al.*, 1995; De Groote & Huyghebaert, 1997; Henry & Miles, 2000; Heaney & Nordin, 2002; Leske & Coon, 2002; Driver *et al.*, 2005; Manangi & Coon, 2008; Vitti & Kebreab, 2010; de Carvalho Mello *et al.*, 2012; Liu *et al.*, 2013). To summarize this interaction; an increase or decrease in dietary Ca or P levels can lead to a decrease in the availability of the other. In poultry, an ideal ratio of Ca:P has been reported to be no wider than 2:1 (Nelson & Walker, 1964; Leske & Coon, 2002; Manangi & Coon, 2008; Hemati Matin *et al.*, 2013) as this ensures the interaction of the two minerals do not influence their availability to the bird. The current study had for both the dilution and summit diets a calculated Ca:P ratio of 1.8:1. Therefore an adverse Ca:P ratio is not the cause for the decrease in P bioavailability. The only logical explanation for the cause of depression in P bioavailability values of the intermediary diets is due to interactions between the calcium salts from MDCP and the PA after mixing the feeds together, which affected the utilization of the available P. This, however, will require further research for confirmation.

3.5.2 Coefficient of total tract digestibility

The second objective was to evaluate the effects of defluorinated PA on nutrient and mineral digestibility coefficients. It is clear that differences ($p \leq 0.05$) in coefficient of total tract digestibility (CTTD) values occur between dietary treatments for P, sodium (Na), iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), and aluminium (Al) (Table 3.5). No significant differences were found for CTTD values of apparent metabolisable energy (AME), protein, fat, fibre, ash, Ca, magnesium (Mg), potassium (K) and boron (Bo). Minerals are divided into two

classes: macro-minerals and micro-minerals with macro-minerals being needed in large amounts and micro-minerals in smaller amounts (Wilson & Beyer, 2000). Macro-minerals that are of importance in the current study include Ca, P, Mg, K, and Na and relevant micro-minerals include Cu, Fe, Mn and Zn. As mentioned in sections 2.6.3 and 3.5.2, interactions occur between all dietary nutrients, including minerals, within a diet. This being said, there is no clear explanation as to the effects of the mineral interactions on absorption and utilization of other minerals. This might be due to mineral interactions having the ability to take place in the feed, intestine and even at a cellular level (Hemati Matin *et al.*, 2013). In a review by Henry and Miles (2000), it was noted that minerals interact with one another more actively through their capability to form chemical bonds. Generally, in broiler production, the mineral requirement is expressed as a mean of the flock's requirements (National Research Council, 1994). Nutritionists therefore ensure sufficient nutrients are available for those birds above the mean by oversupplying by a certain margin, and in doing so give grounds for mineral interactions to take place.

Leg disorders are an on-going issue for the modern broiler that is selected for fast growth. Problems occur due to the weight gain being faster than the rate of bone development, resulting in porous and fragile bones (Rath *et al.*, 2000; Garcia *et al.*, 2013). Phosphorus and Ca together constitute 95% of the bone's mineral matrices, making them the primary nutrients for bone development (Rath *et al.*, 2000). Furthermore, the minerals and nutrients in poultry diets need to be available for absorption such that they can be utilized by the bird, making it vital to quantify the digestibility values of the different sources of both P and Ca. The current study revealed ideal CTTD values for Ca and P in all the diets. However, differences occurred between the treatments for P CTTD. Treatments 100:0 and 0:100 revealed the greatest P CTTD values; however, they did not differ from treatments 75:25 and 25:75 whilst treatment 50:50 had the lowest P CTTD values. With reference to Table 3.2, treatment diet 0:100 also has the highest P bioavailability, allowing the conclusion to be made that the CTTD values of P, together with that of Ca, is sufficient to allow for ideal skeletal bone development and maintenance. Magnesium has a known association with Ca and P (Chicco *et al.*, 1967; Driver *et al.*, 2005; Hemati Matin *et al.*, 2013; Kleyn, 2013). High consumption of Ca or P heightens Mg deficiencies and may reduce the absorption of Mg (Hemati Matin *et al.*, 2013). The current study shows CTTD values of 0.70-0.78% for Mg. Therefore, it is clear that Mg deficiencies did not occur in any of the treatments.

Potassium and Na play part in the body's acid-base equilibrium (Kleyn, 2013), and their electrolytes prove vital to muscle, metabolic and nerve functions (Wilson & Beyer, 2000). Kleyn (2013) states that the chance of K deficiency is very rare as it is difficult to formulate diets with a K level below 0.5%. Furthermore, the body has the ability to regulate absorption of K through tissue protein decomposition which can compensate for the deficiency (Živkov Baloš *et al.*, 2016). Dietary Na levels are recommended to be 0.15% for young birds (Živkov Baloš *et al.*, 2016). However, Murakami *et al.* (1997) suggested that formulation for 21 day old broilers to have 0.25% salt inclusion, which is the source of Na in a diet. Excess levels of both K and Na cause an increase in water uptake, which in turn can result in an electrolyte imbalance (Kleyn, 2013). The current study showed results of sufficient digestibility of both K and Na and no increases in water uptake was evident, illustrating the dietary levels were sufficient. However, the differences seen in CTTD values of Na illustrate a decrease in digestibility when the summit and dilution diets are mixed together. Particular reference must be made to diet 25:75. It is unclear as to the reason for this drop but it may be a cause of interaction between the two inorganic phosphorus sources.

Table 3.5 Mean (± standard error) for coefficient of total intestinal tract digestibility (CTTD) of diets with different levels of the dilution and summit diet fed to broilers.

Parameters	Treatment					p-value
	100:0	75:25	50:50	25:75	0:100	
AME (MJ/kg)	16.721 ± 0.177	16.838 ± 0.087	16.993 ± 0.141	17.194 ± 0.165	16.657 ± 0.118	0.088
Protein	0.894 ± 0.016	0.888 ± 0.008	0.868 ± 0.011	0.872 ± 0.017	0.881 ± 0.014	0.670
Fat	0.952 ± 0.008	0.950 ± 0.006	0.947 ± 0.005	0.953 ± 0.007	0.965 ± 0.006	0.899
Fibre	0.698 ± 0.045	0.713 ± 0.023	0.637 ± 0.030	0.658 ± 0.048	0.763 ± 0.027	0.148
Ash	0.809 ± 0.026	0.819 ± 0.014	0.768 ± 0.020	0.785 ± 0.032	0.832 ± 0.199	0.370
Phosphorus	0.844^{a} ± 0.026	0.798^{ab} ± 0.020	0.699^{b} ± 0.034	0.761^{ab} ± 0.035	0.875^{a} ± 0.015	0.001
Calcium	0.778 ± 0.036	0.797 ± 0.023	0.786 ± 0.027	0.820 ± 0.015	0.847 ± 0.019	0.323
Magnesium	0.784 ± 0.032	0.778 ± 0.018	0.696 ± 0.042	0.742 ± 0.034	0.740 ± 0.030	0.336
Potassium	0.805 ± 0.030	0.785 ± 0.015	0.739 ± 0.032	0.784 ± 0.029	0.757 ± 0.033	0.542
Sodium	0.998^{a} ± 0.001	0.998^{a} ± 0.001	0.997^{ab} ± 0.001	0.995^{b} ± 0.001	0.998^{a} ± 0.001	0.001
Iron	0.997^{a} ± 0.001	0.997^{a} ± 0.001	0.996^{ab} ± 0.001	0.994^{b} ± 0.001	0.996^{a} ± 0.001	0.001
Copper	0.997^{a} ± 0.001	0.997^{a} ± 0.001	0.998^{a} ± 0.001	0.994^{b} ± 0.001	0.998^{a} ± 0.001	<0.001
Zinc	0.997^{a} ± 0.001	0.996^{ab} ± 0.001	0.995^{ab} ± 0.001	0.994^{b} ± 0.001	0.996^{ab} ± 0.001	0.025
Manganese	0.997^{a} ± 0.001	0.997^{a} ± 0.001	0.997^{a} ± 0.001	0.991^{b} ± 0.001	0.996^{a} ± 0.001	0.005
Boron	0.997 ± 0.001	0.997 ± 0.001	0.997 ± 0.001	0.997 ± 0.001	0.997 ± 0.001	0.511
Aluminium	0.997^{a} ± 0.001	0.997^{a} ± 0.001	0.996^{a} ± 0.001	0.994^{b} ± 0.001	0.997^{a} ± 0.001	<0.001

^{a,b} means within rows that have different superscripts differ significantly ($p \leq 0.05$); AME= Apparent metabolisable energy
100:0= 100% dilution diet and 0% summit diet, 75:25= 75% dilution diet and 25% summit diet, 50:50= 50% dilution diet and 50% summit diet, 25:75= 25% dilution diet and 75% summit diet and 0:100= 0% dilution diet and 100% summit diet

No differences were found in protein CTTD values. An earlier study by Hwangbo *et al.* (2009) reported both corn and soya bean meal protein CTTD values to be 98%. The current study made use of the same protein sources in the diets yet the CTTD values were lower than that found by Hwangbo *et al*. (2009). The lower CTTD values in the current study may be a result of protein quality, as protein quality is determined by amino acid (AA) profiles and all meals contain different AA profiles (Boland *et al.*, 2013). For example, a protein may contain all the essential amino acids required. However, if it is deficient in a single AA, then the digestibility of the protein is at its highest just before this essential AA has been depleted.

As mentioned, significant differences in CTTD values occur for Fe, Cu, Zn, Mn, and Al. Dietary treatment 25:75 recorded the lowest CTTD for each of these minerals respectively; however, it was statistically similar to diets 0:100, 50:50 and 75:25 for Zn and diet 50:50 for Fe. Wilson and Beyer (2000) note that these minerals are generally supplied in sufficient concentrations by other ingredients such as soya meal, maize and bone meal. Furthermore, the use of premixes when formulating diets ensure these micro and macro mineral levels are adequate for ideal broiler maintenance and production.

3.6 Conclusion

This study set out to assess the P bioavailability of defluorinated PA as well as the nutrient and mineral digestibility coefficient values of a diet supplemented with defluorinated PA. The bioavailability trial showed significant differences between the dietary treatments. The diet supplemented with PA showed a significantly higher P bioavailability than the rest. The P bioavailability of the intermediary diets showed a significant decrease to those that received only one P supplement. The cause of this was inconclusive; however, it was thought to be due to interactions between the two iP sources. Further research is required to confirm this assumption. Dietary treatments showed to have a significant effect on CTTD values of P, Na, Fe, Cu, Zn, Mn, and Al. It is concluded that defluorinated PA has a desirable P bioavailability and its use in a diet results in competitive CTTD values. Research on the use of defluorinated PA is warranted to assess its effects on broiler production performance and ultimately meat production and quality.

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Chapter 4

The effects of phosphoric acid on broiler production parameters

4.1 Abstract

A 35-day study was conducted to determine the effect of two phosphoric acids (PA) (defluorinated (DF) and defluorinated and desulfonated (DFS) phosphoric acid) as the inorganic phosphate (iP) source on broiler production parameters. Five hundred and forty day-old Cobb500 broilers were randomly allocated to one of nine treatments with six replications per treatment and ten birds per replicate. The control diet (Con) received a commercially available mono-dicalcium phosphate (MDCP) as the inorganic phosphate source; the diet received P inclusion levels based on available phosphorus (aP) levels and was mixed with the grains of the diet. The remaining eight treatment diets received the iP source at one of two inclusion levels that were based on the dietary aP or total phosphorus (tP) levels. Furthermore, they received one of the two PA sources (DF or DFS) which was mixed in either with the grains (G) of the diet, before other micro-ingredients, or last (L) during the mixing of the feed. Therefore, the nine trial diets were 1. Con. 2. aP-DF-G. 3. aP-DF-L. 4. tP-DF-G. 5. tP-DF-L. 6. aP-DFS-G. 7. aP-DFS-L. 8. tP-DFS-G. 9. tP-DFS-L. Live weights and feed intakes were measured weekly until slaughter. Significant ($p \leq 0.05$) differences were found between treatments in live weight, cumulative weight gain, cumulative intake, feed conversion ratio, average daily gain, liveability, protein efficiency ratio and European production efficiency factor. The mixing of the PA into the diet (G or L) showed no effect on production parameters as well as the PA (DF or DFS) used. Diets with P inclusion levels based on aP showed improved results over those with tP inclusion levels regardless of the PA and the mixing method used.

4.2 Introduction

Phosphorus (P) is the second most abundant mineral element within the body and is essential to the growth and development of an animal. It also has the most known functions within the animal body than any other mineral (Mc Donald *et al.*, 2011). Its most vital role being that of formation and maintenance of the skeletal structure and the role it plays in the many metabolic processes which it is involved with. Phosphorus also plays a role in utilization efficiency of feed as well as voluntary feed intake (Bar & Hurwitz, 1984), which are two of the underlying foci of this chapter.

The largest portion of South Africa's broiler diets is plant based, and much of the P found in poultry diets originate from the plant component of the diet (Driver, 2004). A particular portion of this P is bound to phytate in the form of phytic acid (maize= 85% and soya bean= 62%) (Ravindran *et al.*, 1994). Phytate occurs naturally in plants, acting as a primary storage device for P (Hídvégi & Lásztity, 2002). Phytate however, acts as an anti-nutrient (Davies & Reid, 1979), with negative effects on the utilization of P which ultimately limits broiler performance (Sohail & Roland, 1999). Furthermore, the increase in demand of P, from the birds, for skeletal growth and maintenance as well as the attempt to avoid consequences of insufficient P in the diet, have led to the use of inorganic phosphorus supplements, with higher available P (aP) levels, administered in excessive amounts to meet the bird's dietary P requirements (Waldroup, 1999). However, negative environmental impact of the excretion of excessive levels of dietary inorganic phosphorus, has led to limitations of their use in alternative feed formulation strategies (Maguire *et al.*, 2005).

The main P supplements used in animal feeds until the late 1940's were soft rock phosphates and bone meal. However, due to constant development of the broiler industry, there is greater demand for high-P concentration supplements. This has given rise to a number of manufacturing techniques being developed to produce inorganic phosphates with the highest P content possible (Viljoen, 2001). The most common of these being calcium phosphates and defluorinated rock phosphate. Calcium phosphates include monocalcium phosphate (MCP), dicalcium phosphate (DCP) and mono-dicalcium phosphates (MDCP). These are produced by reacting calcium salts with phosphoric acid (PA) (Lima *et al.*, 1997; Waldroup, 1999; Viljoen, 2001). Defluorinated phosphate rock is produced through a reaction between phosphate rock, PA and sodium carbonate (Waldroup, 1999). This process is however very difficult to control and results in great variation in the biological values. This makes the defluorinated rock phosphates less desirable than the calcium phosphates (Waldroup, 1999).

Feed costs for broiler production account for near 70% of the total costs of the business (Teguia & Beynen, 2005) and due to yearly crop yields dropping as a result of global warming (Dar & Gowda, 2013), constraints on the producer due to feed costs will only increase. Furthermore, the increase in demand from the consumer for broiler meat places more pressure on the producer to maintain production. Producers are therefore forced to reduce the cost of feed without compromising performance of the flock (Selle & Ravindran, 2007). The use of

phosphoric acid (PA) as the inorganic phosphorus source may potentially reduce the cost of feed. This can be possible as PA is used to produce the other inorganic phosphate sources and using it directly leads to the elimination of a production process, which may reduce costs. However, the efficiency of PA in broiler production is unknown. Thus the aim of the current study is to assess the efficacy of defluorinated phosphoric acid and defluorinated and desulfonated phosphoric acid in broiler diets. The study objectives were:

i. To determine the effects of the two PAs on production performance of commercial broilers.
ii. To investigate the effects of these PAs at two different inclusion levels based on the dietary total or available phosphorus levels, on production performance.
iii. To evaluate the effects of the two PAs on broiler production performance after being mixed into the feed in one of two sequences.

4.3 Materials and Methods

4.3.1 Birds and housing

Five hundred and forty day-old broiler chicks (Cobb 500) where collected from a commercial hatchery and transported to Mariendahl experimental farm of Stellenbosch University. Vaccination against infectious *bursal* disease (IBD) and Newcastle disease took place at the hatchery. On arrival at Mariendahl, the birds where weighed in groups of ten and randomly allocated to one of 54 cages. Each cage is equipped with two nipple drinkers and a tube feeder. Lighting and humidity and temperature within the house was maintained according to Cobb 500 standards (see Table 3.1 and 3.2 respectively). Water and feed were supplied *ad libitum* throughout the trial. Ethical clearance for the trial was obtained from Stellenbosch University Ethics Board; number SU-ACUM13-00006.

4.3.2 Treatments and experimental diets

The chicks were allocated to one of nine treatments. Treatment diet differences include the supplemented phosphorus (P) source used, how the P source was mixed with the feed and the formulations or P inclusion levels. The control diet (Con), contained a commercially available mono-dicalcium phosphate (MDCP) source with inclusion levels based on available phosphorus (aP) levels and mixed in with the grains of the diet. The remaining eight treatments

had P inclusion levels based on dietary available (aP) or total phosphorus (tP) calculations of the diet. Therefore, the feed formulation were either based on dietary aP or tP levels. The two treatment supplemental phosphorus sources were either a defluorinated (DF) phosphoric acid or a defluorinated and desulfonated (DFS) phosphoric acid. Feeds were mixed in one of two manners; the P mixed into the grain (maize, soya 46 and full fat soya) (G) of the feed, therefore added before any of the micro ingredients, or the P was added last (L) after all the other ingredients had sufficient time to be thoroughly distributed throughout the feed. Concise treatment explanations are available in Table 4.1. The feed was allocated as follows; 900g starter diet (Table 4.2 and 4.3), 1200g grower diet (Table 4.4 and 4.5) and finisher until slaughter (Table 4.6 and 4.7). The different diets were all mixed at Mariendahl experimental farm, Stellenbosch, and were fed as mash.

Table 4.1 The dietary treatment descriptions used throughout the trial.

Treatment	Description
Con	Control, formulated for available phosphorus, using MDCP which was mixed with the grains
aP-DF-G	Formulated for available phosphorus using defluorinated PA which was mixed with the grains
aP-DF-L	Formulated for available phosphorus using defluorinated PA which was mixed in last at the end of the mixing process
tP-DF-G	Formulated for total phosphorus using defluorinated PA which was mixed with the grains
tP-DF-L	Formulated for total phosphorus using defluorinated PA which was mixed in last at the end of the mixing process
aP-DFS-G	Formulated for available phosphorus using defluorinated and desulfonated PA which was mixed with the grains
aP-DFS-L	Formulated for available phosphorus using defluorinated and desulfonated PA which was mixed in last at the end of the mixing process
tP-DFS-G	Formulated for total phosphorus using defluorinated and desulfonated PA which was mixed with the grains
tP-DFS-L	Formulated for total phosphorus using defluorinated and desulfonated PA which was mixed in last at the end of the mixing process

Table 4.2 Starter diet ingredient composition (%) used during the trial.

	Available Phosphorus			Total Phosphorus	
Ingredients	**Con**	**DF**	**DFS**	**DF**	**DFS**
Maize	38.37	38.54	38.54	39.87	39.86
Soybean full fat	44.52	44.17	44.17	41.45	41.47
Soybean 46	12.03	12.29	12.29	14.23	14.21
L-lysine HCl	0.32	0.32	0.32	0.32	0.32
DL methionine	0.43	0.43	0.43	0.42	0.42
L-threonine	0.09	0.09	0.09	0.09	0.09
Vit+min premix*	0.25	0.25	0.25	0.25	0.25
Limestone	1.53	2.50	2.50	2.51	2.51
Salt	0.24	0.24	0.24	0.23	0.23
MDCP	2.06				
Sodium bicarbonate	0.18	0.17	0.17	0.18	0.17
Oil - sunflower					
DF Phosphoric acid		1.02		0.46	
DFS Phosphoric acid			1.02		0.47

*Vitamin and minerals premix
MDCP: mono-dicalcium phosphate
Con: control diet
DF: defluorinated phosphoric acid
DFS: defluorinated and desulfonated phosphoric acid

Table 4.3 Starter diet calculated nutritional values.

		Available Phosphorus			Total Phosphorus	
Nutritional Value	**Units**	**Con**	**DF**	**DFS**	**DF**	**DFS**
AME	MJ/kg	12.82	12.82	12.82	12.82	12.82
Crude protein	%	26.00	26.00	26.00	26.00	26.00
Dry matter	%	89.04	89.03	89.03	88.94	88.94
Lysine	%	1.76	1.75	1.75	1.75	1.75
Methionine	%	0.79	0.79	0.79	0.79	0.79
Threonine	%	1.09	1.09	1.09	1.09	1.09
Tryptophan	%	0.31	0.31	0.31	0.31	0.31
Isoleucine	%	1.19	1.19	1.19	1.19	1.19
Leucine	%	2.14	2.14	2.14	2.15	2.15
Ash	%	4.91	5.86	5.86	5.86	5.86
Crude fibre	%	3.89	3.89	3.89	3.87	3.89
Crude fat	%	9.88	9.82	9.82	9.40	9.40
Calcium	%	1.05	1.05	1.05	1.05	1.05
Phosphorous	%	0.96	0.68	0.68	0.55	0.55
Available phosphorous	%	0.50	0.50	0.50	0.28	0.28

AME: Apparent metabolisable energy
Con: control diet
DF: defluorinated phosphoric acid
DFS: defluorinated and desulfonated phosphoric acid

Table 4.4 Grower diet ingredient composition (%) used during the trial.

	Available Phosphorus			Total Phosphorus	
Ingredients	**Con**	**DF**	**DFS**	**DF**	**DFS**
Maize	50.04	50.07	50.07	50.29	50.28
Soybean full fat	21.76	22.52	22.52	28.04	27.98
Soybean 46	19.90	19.31	19.31	15.05	15.09
L-lysine HCl	0.20	0.12	0.12	0.19	0.19
DL methionine	0.37	0.37	0.37	0.37	0.37
L-threonine	0.11	0.11	0.11	0.11	0.11
Vit+min premix*	0.25	0.25	0.25	0.25	0.25
Limestone	1.31	2.18	2.18	2.17	2.17
Salt	0.25	0.25	0.25	0.25	0.25
MDCP	1.84				
Sodium bicarbonate	0.16	0.16	0.16	0.15	0.15
Oil - sunflower	3.81	3.68	3.68	2.70	2.71
DF phosphoric acid		0.91		0.43	
DFS phosphoric acid			0.91		0.43

*Vitamin and minerals premix
Con: control diet
MDCP: mono-dicalcium phosphate
DF: defluorinated phosphoric acid
DFS: defluorinated and desulfonated phosphoric acid

Table 4.5 Grower diet calculated nutritional values.

		Available Phosphorus			Total Phosphorus	
Nutritional Value	**Units**	**Con**	**DF**	**DFS**	**DF**	**DFS**
AME	MJ/kg	13.48	13.47	13.47	13.46	13.46
Crude protein	%	22.05	22.07	22.07	22.16	22.16
Dry matter	%	88.90	88.90	88.90	88.76	88.76
Lysine	%	1.38	1.38	1.38	1.38	1.38
Methionine	%	0.69	0.69	0.69	0.69	0.69
Threonine	%	0.95	0.95	0.95	0.95	0.95
Tryptophan	%	0.26	0.26	0.26	0.26	0.26
Isoleucine	%	0.99	0.99	0.99	0.99	0.99
Leucine	%	1.90	1.90	1.90	1.91	1.91
Ash	%	4.17	5.02	5.02	5.04	5.04
Crude fibre	%	3.29	3.31	3.31	3.41	3.40
Crude fat	%	10.00	10.00	10.00	10.00	10.00
Calcium	%	0.90	0.90	0.90	0.90	0.90
Phosphorous	%	0.86	0.61	0.60	0.50	0.50
Available phosphorous	%	0.45	0.45	0.45	0.26	0.26

AME: Apparent metabolisable energy
Con: control diet
DF: defluorinated phosphoric acid
DFS: defluorinated and desulfonated phosphoric acid

Table 4.6 Finisher diet ingredient composition (%) used during the trial.

	Available Phosphorus			Total Phosphorus	
Ingredients	**Con**	**DF**	**DFS**	**DF**	**DFS**
Maize	57.10	56.99	56.85	56.99	56.98
Soybean full fat	15.00	17.73	17.73	21.13	21.05
Soybean 46	18.90	16.86	16.86	14.24	14.30
L-lysine HCl	0.20	0.20	0.20	0.19	0.19
DL methionine	0.32	0.32	0.32	0.32	0.32
L-threonine	0.10	0.10	0.10	0.10	0.10
Vit+min premix*	0.25	0.25	0.25	0.25	0.25
Limestone	1.29	2.09	2.09	2.08	2.08
Salt	0.24	0.25	0.25	0.03	0.03
MDCP	1.71				
Sodium bicarbonate	0.17	0.17	0.17	0.16	0.16
Oil - sunflower	4.72	4.35	4.35	3.75	3.76
DF phosphoric acid		0.84		0.55	
DFS phosphoric acid			0.84		0.55

*Vitamin and minerals premix
Con: control diet
MDCP: mono-dicalcium phosphate
DF: defluorinated phosphoric acid
DFS: defluorinated and desulfonated phosphoric acid

Table 4.7 Finisher diet calculated nutritional values.

		Available Phosphorus			Total Phosphorus	
Nutritional Value	**Units**	**Con**	**DF**	**DFS**	**DF**	**DFS**
AME	MJ/kg	13.72	13.71	13.71	13.71	13.71
Crude protein	%	19.64	19.69	19.69	19.74	19.74
Dry matter	%	88.73	88.70	88.70	88.61	88.61
Lysine	%	1.21	1.21	1.21	1.21	1.21
Methionine	%	0.61	0.61	0.61	0.61	0.61
Threonine	%	0.84	0.84	0.84	0.85	0.85
Tryptophan	%	0.22	0.22	0.22	0.22	0.22
Isoleucine	%	0.87	0.87	0.87	0.87	0.87
Leucine	%	1.75	1.75	1.75	1.76	1.76
Ash	%	3.83	4.63	4.63	4.64	4.64
Crude fibre	%	3.03	3.07	3.07	3.13	3.13
Crude fat	%	9.91	10.00	10.00	10.00	10.00
Calcium	%	0.85	0.85	0.85	0.85	0.85
Phosphorous	%	0.80	0.56	0.56	0.50	0.50
Available phosphorous	%	0.42	0.42	0.42	0.30	0.31

AME: Apparent metabolisable energy
Con: control diet
DF: defluorinated phosphoric acid
DFS: defluorinated and desulfonated phosphoric acid

4.3.3 Data Collection

All birds were weighed at placement and weekly thereafter until slaughter at 35 days of age. Cage weight was recorded and an average weight per bird was calculated, correcting for mortalities. Mortalities were recorded twice daily with all dead birds being weighed. Feed consumption per pen was calculated weekly until slaughter, making sure to correct for mortalities. Live weights and feed remaining were used for calculations of weekly feed intake, feed conversion ratio (FCR) (equation 4.1), cumulative feed intake, average daily gain (ADG), protein efficiency ratio (PER) (equation 4.2) and European production efficiency factor (EPEF)

(equation 4.3). European production efficiency factor takes into account liveability, which is the percentage of birds surviving until slaughter expressed as a percentage of birds placed.

The formulae used for calculations of coefficients and ratios are as follows:

Equation 4.1

$$\text{Feed conversion ratio} = \frac{\text{Cumulative feed intake (g)}}{\text{Average live weight gain per chick (g)}}$$

Equation 4.2

$$\text{Protein Efficiency Ratio} = \frac{\text{Weight Gain (g)}}{(\text{Weekly Feed Intake (g)} \times \text{Protein \%})/100}$$

Equation 4.3

$$\text{European production efficacy factor} = \frac{\text{Liveability (\%)} \times \text{Live weight at slaughter (g)}}{\text{Age (days)} \times \text{Feed conversion ratio}} \times 100$$

4.4 Statistical analysis

Statistical analysis was performed using STATISTICA (Dell Inc., 2016). The assumptions for normality and homoscedasticity were investigated first using Levene's test and thereafter further analysis were performed. A significance level of $p \leq 0.05$ was used. Further tests were done by one-way analysis of variance (ANOVA) with the Bonferroni LSD *post hoc* test. Average daily gain (ADG) was calculated by means of simple linear regression with change in weight over time. The subsequent regression function was taken as the ADG and these were compared between treatments.

4.5 Results and discussion

4.5.1 Live weight and cumulative weight gain

The effects of defluorinated PA and defluorinated and desulfonated PA on live weights and cumulative weight gain are depicted in Tables 4.8 and 4.9. No differences ($p>0.05$) were found at hatch (day 0) for live weights across any treatments signifying no differences in weight at the start of the trial. However, on day 7 significant differences between the dietary treatment live weights were found, and for every weekly weighing there after until the end of the trial

(Table 4.8). On day 7, treatment aP-DF-G had the highest mean live weight and remained the highest throughout the trial. On day 14, a trend is noticed between treatment diets formulated for aP (Con, aP-DF-G, aP-DF-L, aP-DFS-G and aP-DFS-L) and treatment diets formulated for tP (tP-DF-G, tP-DF-L, tP-DFS-G and tP-DFS-L). Those diets formulated for aP were seen to have consistently higher mean live weights than those formulated for tP, and this trend is evident from day 14 through to day 35; Figure 4.1 illustrates the mean weekly gain in live weight between all the dietary treatments (growth curve). However, Figure 4.2 may be a better representation of the trend previously referred to. This graph has been made such that the control stands alone and all the diets formulated for aP are represented by a single line as well as all those formulated for tP. It is clear that by day 14 the birds fed the control and diets formulated for aP had begun to gain weight faster than those fed diets formulated for tP. At day 35, there is significant difference between all the diets formulated for aP and those for tP (as seen by use of the superscripts in Table 4.8) and both Figure 4.1 and 4.2 illustrate these differences. Significant differences in cumulative weight gain was found throughout the trial. Similar to the live weights results, dietary treatment aP-DF-G recorded the highest cumulative weight gains throughout the trial. Furthermore, a trend is noticeable between diets formulated for aP and tP, and again those formulated for aP had the higher cumulative weight gains.

On day 21, the birds which were fed diets formulated for aP were found to have a 19.3% greater mean live weight than those formulated for tP, irrespective of the PA used and the method in which the PA was added to the feed (Table 4.8). The diets formulated according to dietary aP levels had 0.50% aP and those formulated according to dietary tP levels have 0.28% aP. Shaw *et al.* (2010) reported a 52.9% increase in body weight on day 21 between birds fed diets with non-phytate phosphorus (npP) levels of 0.45% (standard P levels) and 0.25% (low P levels). The term npP refers to the amount of P plant source that is not bound to phyate and therefore is available to the bird. The cause of these differences between the two studies may be partially due to the difference in Ca:P ratios. The current study has Ca:P ratios between diets formulated for aP and tP of 2.1:1 and 3.8:1, respectively. That reported by Shaw *et al.* (2010) show the Ca:P ratios to be 3.04:1 for the diets with standard P levels and 5.72:1 for the diets with low P levels. Inaccurate ratios of Ca and P has been reported to decrease P utilization (Harrold *et al.*, 1983; Mc Donald *et al.*, 2011). The adverse ratios, generally greater dietary Ca levels, reduce P availability by forming insoluble calcium phosphates in the gastrointestinal tract (Heaney & Nordin, 2002; Mc Donald *et al.*, 2011). Therefore, as the levels of aP are further decreased, Ca

levels increase further, creating wider ratios which affects P utilization and so overall development and growth is affected.

No significant differences were seen between the types of PA used provided their inclusion levels were the same. An assumption could be made that the diets which had the PA included to the grains have greater mean weights. However, after closer inspection this assumption is ruled out as the level of significance for treatments with the PA included to the grains are not different from those with the PA included last. Therefore, the type of PA and the method of mixing the PA into the feed had no effect on live weight and cumulative weight gain.

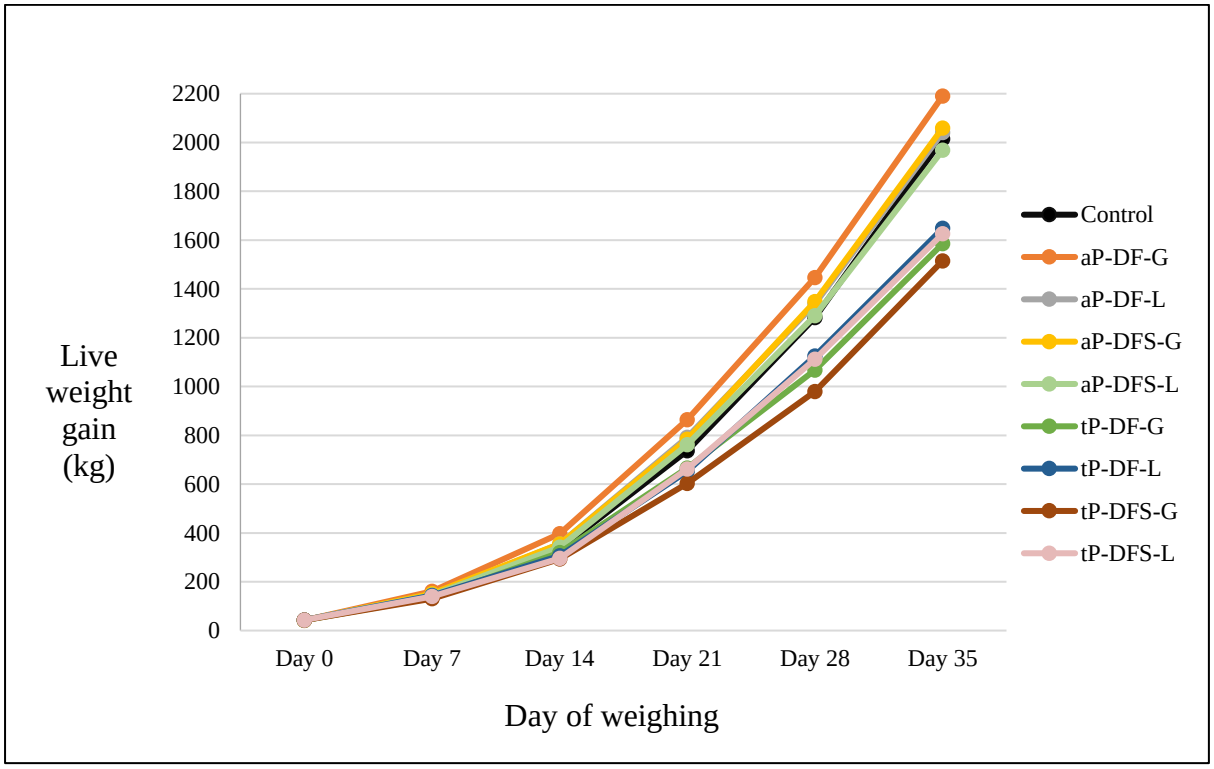

Figure 4.1 Mean live weight of birds fed the dietary treatments from placement to slaughter.

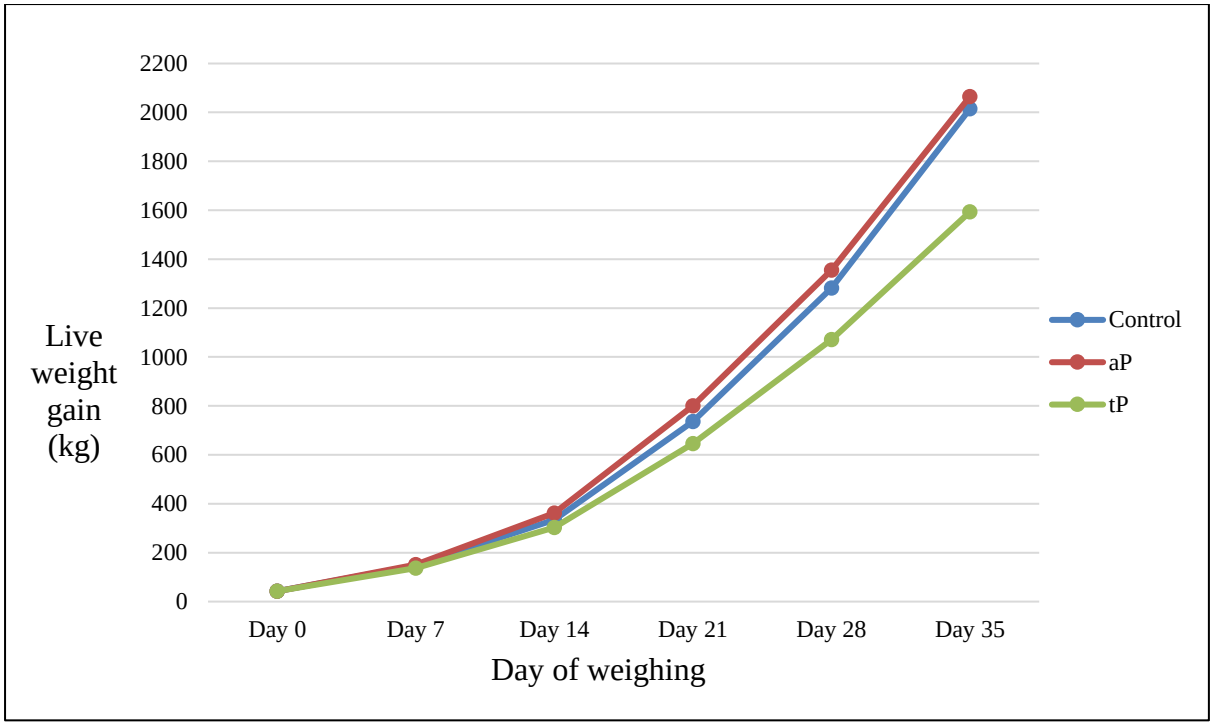

Figure 4.2 Mean live weight of birds fed the control diet and diets that received P inclusion based on the dietary available phosphorus (aP) levels and dietary total phosphorus (tP) levels.

Table 4.8 Mean (± standard error) live weights of birds reared from hatch to day 35 on diets with different phosphorus sources at different P inclusion levels which were mixed to the feed in one of two manners.

Treatment	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35
Con	42.51 ± 0.42	146.92^{bc} ± 1.52	335.05^{ab} ± 11.55	736.29^{bcd} ± 17.15	1282.57^{b} ± 21.13	2014.62^{a} ± 34.20
aP-DF-G	42.00 ± 1.07	160.38^{a} ± 1.98	397.30^{a} ± 10.70	863.75^{a} ± 20.02	1446.58^{a} ± 17.81	2190.54^{a} ± 36.97
aP-DF-L	41.94 ± 0.62	146.03^{bc} ± 3.55	354.83^{ab} ± 9.76	792.04^{ab} ± 17.10	1338.43^{ab} ± 25.98	2040.78^{a} ± 36.23
tP-DF-G	42.22 ± 0.51	134.90^{cd} ± 3.35	319.86^{b} ± 19.60	665.76^{cde} ± 12.68	1067.27^{c} ± 25.17	1584.98^{b} ± 41.66
tP-DF-L	42.97 ± 0.62	143.01^{bcd} ± 2.56	305.76^{b} ± 14.17	653.22^{de} ± 20.77	1125.18^{c} ± 31.81	1647.70^{b} ± 47.68
aP-DFS-G	41.66 ± 0.80	151.39^{ab} ± 2.83	355.27^{ab} ± 16.10	784.21^{ab} ± 27.06	1348.40^{ab} ± 30.91	2059.38^{a} ± 54.33
aP-DFS-L	42.60 ± 0.54	145.60^{bc} ± 1.97	342.22^{ab} ± 20.42	761.42^{bc} ± 34.96	1290.14^{b} ± 43.96	1968.54^{a} ± 72.49
tP-DFS-G	42.47 ± 0.62	130.63^{d} ± 2.96	292.65^{b} ± 18.50	602.31^{e} ± 14.46	979.60^{c} ± 34.20	1515.17^{b} ± 52.22
tP-DFS-L	43.07 ± 0.21	139.95^{bcd} ± 2.10	295.49^{b} ± 5.96	661.76^{cde} ± 16.23	1112.08^{c} ± 39.49	1625.49^{b} ± 42.66
p-value	0.82	<0.01	<0.01	<0.01	<0.01	<0.01

^{a,b} means within columns that have different superscripts differ significantly ($p \leq 0.05$)
Con: Control diet formulated for available phosphorus, using mono-dicalcium phosphate, mixed with the grains
aP-DF-G: Formulated for available phosphorus using de-fluorinated PA, mixed with the grains.
aP-DF-L: Formulated for available phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
tP-DF-G: Formulated for total phosphorus using de-fluorinated PA, mixed with the grains.
tP-DF-L: Formulated for total phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
aP-DFS-G: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
aP-DFS-L: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.
tP-DFS-G: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
tP-DFS-L: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.

Table 4.9 Mean (± standard error) cumulative weight gain of birds reared from hatch to day 35 on diets with different phosphorus sources at different P inclusion levels which were mixed to the feed in one of two manners.

Treatment	Day 0-7	Day 0-14	Day 0-21	Day 0-28	Day 0-35
Con	104.41^{bc} ± 1.49	292.54^{ab} ± 11.28	593.22^{bcd} ± 20.87	1157.41^{a} ± 53.59	1887.98^{a} ± 85.11
aP-DF-G	118.38^{a} ± 1.93	355.30^{a} ± 10.07	821.75^{a} ± 19.40	1404.58^{a} ± 18.06	2148.20^{a} ± 30.43
aP-DF-L	104.10^{bc} ± 3.43	287.11^{ab} ± 10.03	702.08^{ab} ± 16.10	1303.60^{a} ± 30.51	1980.55^{a} ± 40.47
tP-DF-G	91.85^{cd} ± 3.36	275.90^{b} ± 19.55	620.72^{cd} ± 14.30	1032.99^{c} ± 25.10	1418.78^{b} ± 27.27
tP-DF-L	98.85^{bcd} ± 3.36	263.17^{b} ± 4.47	605.84^{d} ± 18.48	1089.62^{bc} ± 37.17	1578.22^{b} ± 68.51
aP-DFS-G	108.72^{ab} ± 3.43	314.32^{ab} ± 15.46	743.27^{ab} ± 26.40	1307.46^{a} ± 30.14	2004.45^{a} ± 51.45
aP-DFS-L	103.00^{bc} ± 1.91	299.62^{ab} ± 20.18	718.20^{bc} ± 35.27	1257.28^{ab} ± 36.88	1922.86^{a} ± 59.45
tP-DFS-G	88.16^{d} ± 3.23	250.18^{b} ± 18.28	555.98^{d} ± 14.98	938.27^{c} ± 36.06	1416.21^{b} ± 42.88
tP-DFS-L	97.17^{bcd} ± 1.80	254.37^{b} ± 6.62	620.64^{cd} ± 17.18	1066.0^{c} ± 35.28	1566.51^{b} ± 47.38
p-value	<0.01	<0.01	<0.01	<0.01	<0.01

^{a,b} means within columns that have different superscripts differ significantly ($p \leq 0.05$)
Con: Control diet formulated for available phosphorus, using mono-dicalcium phosphate, mixed with the grains
aP-DF-G: Formulated for available phosphorus using de-fluorinated PA, mixed with the grains.
aP-DF-L: Formulated for available phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
tP-DF-G: Formulated for total phosphorus using de-fluorinated PA, mixed with the grains.
tP-DF-L: Formulated for total phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
aP-DFS-G: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
aP-DFS-L: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.
tP-DFS-G: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
tP-DFS-L: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.

4.5.2 Cumulative intake

The results of cumulative feed intake are shown in Table 4.10. On day 0-7 and day 0-14 there are no significant differences seen in cumulative feed intake. Differences in cumulative feed intake are however evident on days 0-21, 0-28 and 0-35 ($p<0.01$). Treatment aP-DF-G has the highest intake for days 0-7, -14 and -21. Thereafter, birds fed the Con treatment diet had the highest intake (day 0-28 and 0-35). However, there is no difference between the Con and aP-DF-G as well as the other diets formulated for aP on these days. Chickens which received treatment tP-DFS-G had the lowest intake throughout the trial.

Viveros *et al.* (2002) reports differences ($p\leq0.05$) in the broiler feed intake of diets with different npP levels. It was reported that birds fed diets with npP levels of 0.35 and 0.22% had significantly greater feed consumption than those fed diets with npP levels of 0.27 and 0.14%, respectively. These findings are similar to that found in the current study as diets which received PA inclusion levels based on aP (aP= 0.42-0.50%) had higher intake than the tP (aP= 0.26-0.30%) diets. These two studies are not comparable as one refers to the npP levels and the other aP levels, however the application where the higher P levels results in higher intake is the same. It is also clear that the type of PA used and the method of incorporating it into the diet had no significant effect on intake provided the diets P inclusion levels are the same.

Suttle (2010) states that there is a correlation between appetite loss and phosphorus deprivation. This seems to be the case on days 0-21, 0-28 and 0-35. More particularly in the last two weeks of the trial (day 0-28 and 0-35). The treatment diets with P inclusion levels based on tP levels (aP= 0.26-0.30%) resulted in lower cumulative intakes than those with P inclusion levels based on aP (aP= 0.42-0.45%). Therefore, as theorised by Suttle (2010), loss of appetite and subsequent P deprivation initially started between days 14 and 21, as no significant differences in intake occurred between days 0-7 and -14. Although the p-value for day 0-14 ($p= 0.086$) indicated a tendency to differ; this could indicate that the initial onset of deprivation may have occurred earlier.

Table 4.10 Mean (± standard error) cumulative intake (g) of birds reared from hatch to day 35 on diets with different phosphorus sources at different P inclusion levels which were mixed to the feed in one of two manners.

Treatment	Day 0-7	Day 0-14	Day 0-21	Day 0-28	Day 0-35
Con	139.78 ± 3.35	323.78 ± 11.57	1032.68^{abc} ± 29.28	2077.46^{a} ± 66.74	3236.58^{a} ± 69.05
aP-DF-G	151.27 ± 3.70	372.53 ± 11.22	1138.77^{a} ± 40.92	1997.92^{a} ± 40.66	3143.19^{a} ± 77.32
aP-DF-L	146.07 ± 3.12	338.27 ± 5.89	1091.70^{ab} ± 16.65	1956.57^{a} ± 30.39	3032.64^{a} ± 66.29
tP-DF-G	141.17 ± 7.48	332.00 ± 17.63	967.36^{abc} ± 30.60	1661.49^{cd} ± 28.89	2242.41^{b} ± 74.97
tP-DF-L	142.22 ± 8.34	325.51 ± 17.57	947.37^{bc} ± 24.29	1673.32^{bcd} ± 40.40	2383.38^{b} ± 63.16
aP-DFS-G	141.78 ± 6.35	337.03 ± 11.26	1073.03^{ab} ± 21.94	1919.35^{ab} ± 36.82	3019.67^{a} ± 89.52
aP-DFS-L	143.15 ± 4.84	325.08 ± 18.02	1028.72^{abc} ± 64.45	1850.05^{abc} ± 75.41	2877.85^{a} ± 34.84
tP-DFS-G	130.53 ± 6.77	284.03 ± 25.05	884.62^{c} ± 44.49	1553.02^{d} ± 60.15	2242.43^{b} ± 96.95
tP-DFS-L	142.75 ± 6.21	305.67 ± 27.96	970.44^{abc} ± 31.42	1679.33^{bcd} ± 65.10	2450.90^{b} ± 96.49
p-value	0.56	0.09	<0.01	<0.01	<0.01

^{a,b} means within columns that have different superscripts differ significantly ($p \leq 0.05$)
Con: Control diet formulated for available phosphorus, using mono-dicalcium phosphate, mixed with the grains
aP-DF-G: Formulated for available phosphorus using de-fluorinated PA, mixed with the grains.
aP-DF-L: Formulated for available phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
tP-DF-G: Formulated for total phosphorus using de-fluorinated PA, mixed with the grains.
tP-DF-L: Formulated for total phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
aP-DFS-G: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
aP-DFS-L: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.
tP-DFS-G: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
tP-DFS-L: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.

4.5.3 Feed conversion ratio

Results of feed conversion ratios (FCR) for the current study are depicted in Table 4.11. The feed conversion ratio is a measure of how efficiently the animal uses the nutrients available to it. No significant difference in FCR was seen at week 2 (day 0-14) and 5 (day 0-35), however, treatment differences ($p \leq 0.05$) are seen at week 1 (day 0-7), 3 (day 0-21) and 4 (day 0-28). In week 1, birds fed the aP-DF-G treatment diet recorded the best (lowest) FCR and this treatment continued to have the best FCR through the trial. In week 3, the Con treatment diet had the highest FCR and treatment tP-DFS-G recorded the highest FCR in week 4. The remainder of all the treatments were intermediary for both weeks.

Butcher and Nilipour (2002) reported a FCR value of no more than 1.85 is essential for broiler production to be normal and a lower FCR is more beneficial and indicates positive results. Therefore, the feed in the current study was used efficiently throughout the trial as all FCR values are all below 1.85. The diets with P inclusion levels based on aP resulted in lower, therefore better, FCR values than the Con diet throughout the trial. The control diet closely resembled commercially available diets therefore the PA's prove to be an effective iP sources in maintaining a good FCR.

Table 4.11 Mean (± standard error) feed conversion ratios of birds reared from hatch to day 35 on diets with different phosphorus sources at different P inclusion levels which were mixed to the feed in one of two manners.

Treatment	Day 0-7	Day 0-14	Day 0-21	Day 0-28	Day 0-35
Con	1.37^{ab} ± 0.03	1.11 ± 0.03	1.60^{a} ± 0.03	1.61^{ab} ± 0.04	1.59 ± 0.02
aP-DF-G	1.28^{b} ± 0.03	1.05 ± 0.02	1.38^{c} ± 0.02	1.42^{c} ± 0.03	1.46 ± 0.02
aP-DF-L	1.41^{ab} ± 0.05	1.09 ± 0.04	1.47^{abc} ± 0.03	1.50^{bc} ± 0.02	1.53 ± 0.02
tP-DF-G	1.54^{a} ± 0.05	1.21 ± 0.05	1.56^{ab} ± 0.03	1.61^{ab} ± 0.01	1.58 ± 0.04
tP-DF-L	1.43^{ab} ± 0.05	1.24 ± 0.09	1.57^{ab} ± 0.04	1.54^{abc} ± 0.04	1.52 ± 0.04
aP-DFS-G	1.30^{b} ± 0.04	1.08 ± 0.04	1.45^{abc} ± 0.03	1.47^{bc} ± 0.01	1.51 ± 0.01
aP-DFS-L	1.39^{ab} ± 0.05	1.10 ± 0.06	1.43^{bc} ± 0.04	1.47^{bc} ± 0.05	1.49 ± 0.04
tP-DFS-G	1.48^{ab} ± 0.03	1.13 ± 0.06	1.59^{ab} ± 0.05	1.66^{a} ± 0.01	1.58 ± 0.04
tP-DFS-L	1.47^{ab} ± 0.08	1.20 ± 0.09	1.56^{ab} ± 0.02	1.57^{ab} ± 0.03	1.57 ± 0.04
p-value	<0.01	0.25	<0.01	<0.01	0.06

^{a,b} means within columns that have different superscripts differ significantly ($p \leq 0.05$)
Con: Control diet formulated for available phosphorus, using mono-dicalcium phosphate, mixed with the grains
aP-DF-G: Formulated for available phosphorus using de-fluorinated PA, mixed with the grains.
aP-DF-L: Formulated for available phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
tP-DF-G: Formulated for total phosphorus using de-fluorinated PA, mixed with the grains.
tP-DF-L: Formulated for total phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
aP-DFS-G: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
aP-DFS-L: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.
tP-DFS-G: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
tP-DFS-L: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.

4.5.4 Average daily gain, PER, EPEF and liveability

The dietary treatment effects on average daily gain (ADG), European production efficiency ratio (EPER), protein efficiency ratio (PER) and liveability are presented in Table 4.12. Significant differences were found in all these scientific calculations. Dietary treatment aP-DF-G reported the highest ADG, EPEF, PER and liveability. These calculations take into account live weight, weight gain and feed intake, all of which treatment aP-DF-G had performed the best. Also, all the diets formulated for aP resulted in better values than those formulated for tP.

The mortality rate of 6.5% for the current study is much higher than that which is acceptable for a flock (2%). However, 1.45% of the deaths were unexpectedly recorded by the control treatment in the beginning of the grower phase. After *post-mortem* inspection on the first two

of these birds was completed, it was found that the deaths were caused by sodium (Na) poisoning. This occurred at the beginning of the grower feeding period on day 14. The birds received the grower feed at approximately 08:00 and an issue was noticed the next morning at 06:00 and after realisation of the poisoning the feed was removed at approximately 10:00 am. This means the birds had access to the high Na feed only for approximately 26 hours. Thereafter birds only had access to water for approximately five hours to allow for 'flushing' of the birds system whilst new feed was being mixed and once the new feed was completed the birds were given the feed immediately. The birds were then monitored closely for the remainder of the day to ensure no further issues occurred. This might be seen as something which could affect the birds overall growth and therefore, the statistical analysis for all the data obtained within this chapter was rerun without the control diet's data being included in the analysis. The rerun showed very little difference between the two sets of statistical analysis (Addendum A) and so it was concluded that this error had no effect on the overall production of the birds fed the control diet and the control could still be compared to as a commercial broiler diet yielding results that would normally be obtained in industry. A further 3.52% of the deaths occurred in the last week (week 5) of the trial. These deaths came only from the treatment diets that had P inclusion levels based on the dietary tP levels. Of this 3.52%, 0.95% were culled due to leg disorders and 2.57% of the deaths were due to morbidity. It has been reported that excessive undersupply of P can result in high mortalities (Waldroup, 1999). Furthermore, it was mentioned in section 4.5.2 that the onset of P deprivation occurred in week 3 of the trial. The conclusion can therefore be made that during week 5 the P deprivation had become fatal.

To obtain the best production efficiency from a flock, the following minimum values should be adhered to: at 35 days, a live slaughter weight between 1.5-2 kg, with a FCR less than 1.85, ADG greater than 50g and finally an EPEF value greater than 260 (Butcher & Nilipour, 2002). All treatments' live weight and FCR values are better than the minimum stated by Butcher and Nilipour (2002). Although all the diets formulated for tP are on the borderline of the minimum for live weight. All diets formulated for aP meet minimum requirements for ADG and EPEF whereas those formulated for tP do not meet these minimum standards, except for treatments tP-DFS-L and tP-DF-L, which are slightly above the minimum EPEF value. The calculation of EPEF value includes the liveability of the flock. Therefore, the low liveability seen in treatments formulated for tP, due to P deprivation, may be cause for the low EPEF values for these treatments.

The PER is the amount of weight gained for every unit of protein intake, and a PER value below 1.5 is indicative of low dietary protein quality as well as low protein utilization (Johnson & Parsons, 1997). The current study shows the treatments ($p \leq 0.05$) affected PER, with the control treatment having the lowest PER value (PER= 2.87). However, all PER values obtained indicate that the dietary protein was utilized efficiently.

Table 4.12 Mean (± standard error) average daily gain (ADG), European production efficiency ratio (EPER), protein efficiency ratio (PER) and liveability of birds reared from hatch to day 35 on diets formulated for total or available phosphorus, using phosphoric acid as the phosphorus source and different mixing sequences

Treatment	ADG (g)	EPEF	PER	Liveability (%)
Con	57.56^{b} ± 0.98	306.65^{c} ± 15.38	2.87^{e} ± 0.03	85.00^{b} ± 5.00
aP-DF-G	62.59^{a} ± 1.06	428.29^{a} ± 8.19	3.32^{a} ± 0.05	100.00^{a} ± 0.00
aP-DF-L	58.31^{b} ± 1.04	368.19^{b} ± 10.68	3.24^{ab} ± 0.04	96.67^{a} ± 2.11
tP-DF-G	45.29^{c} ± 1.19	236.13^{e} ± 8.70	2.90^{de} ± 0.06	83.33^{b} ± 6.15
tP-DF-L	47.08^{c} ± 1.32	292.61^{c} ± 21.65	3.09^{bc} ± 0.08	93.33^{ab} ± 3.33
aP-DFS-G	58.84^{ab} ± 1.55	384.77^{b} ± 14.47	3.36^{a} ± 0.02	98.33^{a} ± 1.67
aP-DFS-L	56.24^{b} ± 2.07	364.05^{b} ± 13.18	3.16^{bc} ± 0.06	96.67^{a} ± 2.11
tP -DFS-G	43.29^{c} ± 1.49	244.92^{de} ± 24.50	3.01^{cde} ± 0.06	90.00^{ab} ± 5.16
tP-DFS-L	46.44^{c} ± 1.22	278.33^{cd} ± 13.97	3.04^{cd} ± 0.08	93.33^{ab} ± 2.11
p-value	<0.01	<0.01	<0.01	0.02

^{a,b} means within columns that have different superscripts differ significantly ($p \leq 0.05$)
Con: Control diet formulated for available phosphorus, using mono-dicalcium phosphate, mixed with the grains
aP-DF-G: Formulated for available phosphorus using de-fluorinated PA, mixed with the grains.
aP-DF-L: Formulated for available phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
tP-DF-G: Formulated for total phosphorus using de-fluorinated PA, mixed with the grains.
tP-DF-L: Formulated for total phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
aP-DFS-G: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
aP-DFS-L: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.
tP-DFS-G: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
tP-DFS-L: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.

4.6 Conclusion

The current study was implemented to determine the effects of defluorinated phosphoric acid and defluorinated and desulfonated phosphoric acid at two inclusion levels that were mixed into the diet in two different manners on broiler production performance. A distinct

improvement in the treatments with P inclusion levels based on aP is evident in the measurements of live weight, cumulative weight gain, cumulative intake and the calculations of FCR, ADG, EPEF and PER. This is regardless of the PA and mixing method used. The lower liveability in the control (typical of a commercial diet) was due to sodium poisoning which occurred when the birds were first given access to the grower feed. However, P deprivation occurred in the final stages of the trial in the treatments with low P inclusion levels (based on tP) and this deprivation accounts for the majority of the mortalities. The method used when mixing the PA into the diet had no effect on production performance. Diets which used the PA with inclusion levels based on aP showed consistently similar, and in some instances better values in all measurements, except for cumulative intake, when compared to the control diet. As the control diet is based on a diet that is used commercially, the conclusion can be made that both defluorinated PA and defluorinated and desulfonated PA are effective inorganic phosphorus sources that will maintain, and at times improve broiler production performance, on condition that P inclusion levels are sufficient to allow it.

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Chapter 5

Influence of phosphoric acid on carcass characteristics and meat quality

5.1 Abstract

Carcass characteristics and breast and thigh meat quality of broiler chickens fed diets supplemented with either a defluorinated phosphoric acid (DF) or a defluorinated and desulfonated phosphoric acid (DFS) were investigated. The control diet used a standard mono-dicalcium phosphate (MDCP) as the phosphorus source with the inclusion levels calculated according to dietary available phosphorus (aP) levels. A further eight treatment diets were also mixed with one of the respective phosphorus source that was added either to the grains (G) or added last during the mixing of the diets (L). Phosphoric acid (PA) inclusion levels were made according to dietary aP or total phosphorus (tP) levels. Five hundred and forty Cobb 500 broiler chicks were randomly allocated to the treatment diets and slaughtered at 35 days of age. Treatment effects were not significant for the breast and thigh initial and ultimate pH, hue angle, dressing percentage, muscle percentage, bone percentage, skin and fat percentage as well as the portion weights of the breast and thigh. Breast meat colour CIE-Lab measurement (L*, a* and b*) as well as chroma values were affected on the tP-DF-L dietary treatment and was significantly lighter (higher L* values) than the other breasts and recorded significantly higher chroma values. The tP-DFS-L treatment indicated significantly heavier drumstick and wing portion weights. It was concluded that neither of the phosphoric acids nor the method of mixing had negative effects on carcass characteristics or the meat quality of the breast and thigh muscle. However, P inclusions levels did effect live, warm and cold carcass weights.

5.2 Introduction

The poultry industry has seen a trend whereby consumers, who once preferred purchasing the whole bird; now prefer to purchase only the portion of the bird they are willing to eat. These portions are more known in industry as secondary processed products (Dransfield & Sosnicki, 1999; Zhao *et al.*, 2012). Secondary processing, also known as value adding, is a result of the modern lifestyle of higher disposable income and less available time on hand to cook. This has led to consumers being able and willing to pay an extra amount for partially prepared products

(Owens *et al.*, 2010). Due to this change in consumer preference, the industries' marketing strategies have to be re-assessed to account for the change. Much of todays' poultry is being processed into higher value products such as breasts and de-boned pieces (Young *et al.*, 2001).

Meeting the demands of the consumer means the birds need a desirable carcass conformation, as well as larger edible portion yields (Bogosavljevic-Boskovic *et al.*, 2010). The preferred portions are the breast, drumstick (leg), thigh and wing and are represented as a percentage of the carcass weight. The success of the broiler industry depends greatly on the producers' ability to increase the yields of these portions of the carcass (Guerrero-Legarreta, 2010). Producers also need to maintain structural integrity of the bone to ensure this growth can take place, with emphasis on calcium and phosphorus specifically (Angel, 2011). This is due to phosphorus and calcium forming the building blocks of the skeleton (Soares, 1995). Therefore, it is essential to ensure the dietary phosphorus level meets the bird's daily requirements. Much of the dietary P is of plant origin (Van der Klis & Versteegh, 1999). However, this source of P is predominately unavailable to monogastric animals and so their diets need P supplements to alleviate any shortages. Much of these supplements are inorganic phosphates (iP) (Viljoen, 2001). The increase in demand for iP supplements has led to an increase in research and development in this field, illustrating the significance of potentially using phosphoric acid (PA) as an inorganic phosphate source.

Meat quality in general is a complex topic that has various aspects to it. Not only do carcasses need to have good carcass composition and slaughter yields but also need to be aesthetically pleasing to the eye with good sensory and nutritional characteristics (Bogosavljevic-Boskovic *et al.*, 2010). All these aspects add to the quality of the meat; poultry meat included.

The colour of meat is of great importance (Fanatico *et al.*, 2007). It is the first feature a consumer notices when considering to buy a meat product and this is more specific to value added products (Fanatico *et al.*, 2007). When purchasing meat products, a consumer generally has two preferences; appearance (colour) and palatability (Kropf, 1980). Palatability is determined ultimately by the overall meat quality of the product; therefore, due to consumers having little means of determining meat quality, they must make their decision solely based on the appearance of the product.

Considerable development has been made in growth efficiency and portion yields of poultry (Havenstein *et al.*, 2003). However, failure to improve meat quality characteristics has led to defects in the meat products such as DFD (dark, firm and dry) and PSE (pale, soft and exudative) (Souza *et al.*, 2011). These defects are a result of muscle pH changes *post-mortem*. Another phenomenon that is gaining publicity in the poultry industry is woody breast condition (WBC). Woody breast condition is characterised by unappealing tactile defects on the raw breast fillet, which causes it to be firmer than the normal breast fillet, as well as to have lower protein functionality in further processed products (Mudalal *et al.*, 2014; Sihvo *et al.*, 2014). Woody breast condition is said to be associated with the fast growth of the modern broiler (Mutryn *et al.*, 2015). This unappealing appearance of the fillet may have negative effects on the sale potential of the fillet (Kuttappan *et al.*, 2016).

Post-mortem pH changes are vital to maintaining the functional properties of meat, with a sharp drop in pH soon after death being characteristic of PSE meat (Castellini *et al.*, 2008). The ultimate pH of a muscle affects the myoglobin's ability to express the red colour in meat (Souza *et al.*, 2011). The *post-mortem* pH drop is determined by the muscles glycolytic enzyme activity, therefore, the ultimate pH is affected by the muscles glycogen reserves at point of slaughter (Fanatico *et al.*, 2007). Lonergan *et al.* (2003) reported a strong positive correlation between pH and redness (a*) and negative correlation between pH and lightness values of meat.

Little has been reported on the effects of PA on carcass characteristics and the physical measurements of pH and colour of broiler chicken meat. Therefore, this study's objectives were to:

i. Assess the effects of two different types of phosphoric acids on carcass weights, portion weights, dressing percentage, meat pH and colour.
ii. Evaluate the effects of adding the phosphoric acids to the diet at two inclusion levels on carcass weights, portion weights, dressing percentage, meat pH and colour.
iii. Determine the effects of adding the phosphoric acid to the diet at different intervals during the mixing process on carcass weights, portion weights, dressing percentage, meat pH and colour.

5.3 Materials and methods

5.5.1 Birds, housing and experimental procedure

A thorough explanation of the chickens, housing, diets and experimental procedures is found in Chapter 4. Briefly, nine dietary treatments were randomly designed into six replicates per treatment with ten birds per replicate. The first treatment is the control, which received a standard commercial mono-dicalcium phosphate. This was added to the grains during the mixing of the feed (G). Formulations and therefore inclusion levels of the MDCP were calculated according to available phosphorus (aP). The two sources of PA were added either to the grains (maize, soya 46 and full fat soya) during mixing, or added last after all other ingredients had been thoroughly mixed together (L). Inclusion levels of PA were calculated either according to aP or total phosphorus (tP) levels of the diet. This gave rise to a further eight treatment diets:

1. aP-DF-G: Formulated for available phosphorus using de-fluorinated PA, mixed with the grains.
2. aP-DF-L: Formulated for available phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
3. tP-DF-G: Formulated for total phosphorus using de-fluorinated PA, mixed with the grains.
4. tP-DF-L: Formulated for total phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
5. aP-DFS-G: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
6. aP-DFS-L: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.
7. tP-DFS-G: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
8. tP-FDS-L: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.

The respective diets were offered *ad libitum* to day-old broiler chickens. Treatment specifications are explained fully in Table 4.1. From each cage, one bird close to the mean

weight of the birds in the cage was selected for slaughter at day 35. It was argued that this selection would minimise the effect of the Na poisoning in the Control treatment as discussed in the previous chapter and that the birds were representative of their treatments and were all healthy. Broilers were slaughtered according to standard commercial practice at a commercial abattoir. This involved electrical stunning (50-70 volts; 3-5 seconds), followed by exsanguination.

5.5.2 Carcass characteristics and physical measurements

After slaughter, the birds were scalded, defeathered and eviscerated. The initial muscle pH of the right thigh and breast was determined within 15 minutes of slaughter using a portable, calibrated (buffers pH 4.0 and 7.0 at room temperature) Crison pH25 meter. Following initial pH readings, the carcasses were hung in cold storage at 4°C for 24 hours. Ultimate pH was determined in a similar manner to that of the initial pH, however in the left thigh and breast muscles after 24 hours in cold storage.

Live weight, warm carcass and cold carcass weight (24 hrs post mortem) were recorded. The percentile difference between the live weight and the hot carcass weight were used to calculate dressing percentage. Commercial portion yields were determined using a portion cutter and cutting the cold carcass in half. Thereafter, the wings were removed by cutting between the scapula and coracoid. Removal of the thigh and drumstick required an incision above the thigh, cutting behind the pubic bone, toward the acetabulum. The thigh and drumstick were then separated with a perpendicular cut to the joint connecting the two cuts. Weights of the separate portions were recorded using a Mettler PC 4400 scale (Mettler-Toledo, Switzerland). These portion weights, expressed as a percentage relative to the chilled carcass weight, gave the percentage of the component yields.

The right breast portion was skinned and deboned, where after the weights of the muscle, bone and skin and fat were separately recorded for determination of muscle, bone and skin and fat percentage. The muscle was then cut in half and allowed to bloom for 30 minutes at 8 ºC. The meat colour was measured using a CIE-Lab colour meter (BYK-Gerdner GmbH, Gerestried, Germany). Measurements taken were L* (lightness), a* (redness) and b*(yellowness). Triplicate measurements were taken over the exposed meat and the average calculated. The

hue angle and chroma values of the breast was calculated using equations 5.1 and 5.2 respectively.

Equation 5.1

$$\text{Chroma } (C^*) = \sqrt{(a^*)^2+(b^*)^2}$$

Equation 5.2

$$\text{Hue } (h_{ab}) = \tan^{-1}\left(\frac{b^*}{a^*}\right)$$

5.4 Statistical analysis

All statistical analyses were performed using the general linear models procedure in STATISTICA (Dell Inc., 2016). All data were tested for homoscedasticity and normality using Levene's test before any further analysis was performed. If the assumptions were correct ($p>0.05$), a one-way analysis of variance (ANOVA) was performed with Bonferroni LSD *post hoc* test to analyse any differences between the treatments diets.

5.5 Results and Discussion

5.5.1 Carcass characteristics

5.5.1.1 Dressing percentage and carcass component yield

Diet influenced the live weight, as well as warm and cold carcass weights ($p\leq0.05$) of the broilers (Table 5.1). However, even though the selection of the specific birds per replication per diet would have led to these differences (particularly live weight); these differences are still representative of the effect of the diets on the overall growth of the broilers (see Chapter 4). The aP-DF-G treatment group (defluorinated PA with inclusion levels calculated according to aP and mixed with the grains) yielded the highest weights and the tP-DFS-L group the lowest. Portion differences were miniscule between the P sources, provided the P inclusion levels were the same (based on either dietary tP or aP levels). Portions from treatments with P inclusion levels, and therefore formulations, based on dietary available phosphorus levels (aP= 0.42-0.50%) differ significantly (heavier) from those based on dietary total phosphorus levels (aP= 0.26-0.30%) in all weights.

Table 5.1 Mean (± standard error) of live weight and warm and cold carcass weight of broilers reared until 35 days of age on feeds formulated and mixed in different manners using two different phosphoric acids.

Treatment	Live weight (g)	Warm carcass weight (g)	Cold carcass weight (g)
Con	2003.8^{abcd} ± 103.6	1359.4^{abc} ± 67.6	1344.5^{abcd} ± 68.2
aP-DF-G	2323.7^{a} ± 69.6	1607.8^{a} ± 46.2	1593.0^{a} ± 46.1
aP-DF-L	2084.6^{abc} ± 80.8	1433.1^{ab} ± 56.4	1419.3^{ab} ± 56.8
tP-DF-G	1629.5^{de} ± 135.9	1049.7^{d} ± 84.5	1066.0^{de} ± 91.4
tP-DF-L	1833.5^{bcde} ± 55.5	1212.9^{bcd} ± 28.4	1199.5^{bcde} ± 27.6
aP-DFS-G	2031.7^{abc} ± 65.2	1408.6^{abc} ± 50.9	1389.3^{abc} ± 49.6
aP-DFS-L	2121.7^{ab} ± 38.8	1394.7^{abc} ± 54.8	1382.7^{abc} ± 56.2
tP-DFS-G	1703.0^{cde} ± 80.6	1129.8^{cd} ± 58.9	1115.5^{cde} ± 59.7
tP-DFS-L	1544.2^{e} ± 60.8	1004.2^{d} ± 64.2	995.0^{e} ± 63.5
p-value	<0.01	<0.01	<0.01

^{a,b} means within columns that have different superscripts differ significantly ($p \leq 0.05$)
Con: Control diet formulated for available phosphorus, using mono-dicalcium phosphate, mixed with the grains.
aP-DF-G: Formulated for available phosphorus using de-fluorinated PA, mixed with the grains.
aP-DF-L: Formulated for available phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
tP-DF-G: Formulated for total phosphorus using de-fluorinated PA, mixed with the grains.
tP-DF-L: Formulated for total phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
aP-DFS-G: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
aP-DFS-L: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.
tP-DFS-G: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
tP-DFS-L: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.

Dressing percentage is the marketable higher value portion of a carcass and is influenced by visceral growth and muscle growth. No differences in dressing percentage, muscle, bone, skin and fat percentage was found ($p>0.05$) between the treatments (Table 5.2). These findings are similar to those reported by Kozlowski *et al.* (2009), with reference to the effects of dietary phosphorus levels on dressing percentage. However, Çimrin and Demirel, (2008) who tested diets with inadequate available phosphorus (0.20, 0.22, 0.28 and 0.30% aP) to that with adequate available phosphorus (0.44, 0.44, 0.25 and 0.50% aP), found the birds which received diets with adequate aP to have higher ($p \leq 0.05$) dressing percentage. Although these two studies are not comparable to the current study due to both testing different phytases in broiler production, the treatments do correlate with this study with regard to the levels of aP. The

current study recorded aP values for the diets formulated for total and available phosphorus to be 0.26-0.30% and 0.42-0.50% aP respectively.

Table 5.2 Mean (± standard error) dressing percentage and proportion of the muscle, bone, skin and fat of the breast of broilers reared until 35 days of age on feeds formulated and mixed in different manners using two different phosphoric acids.

Treatment	Dressing Percentage (%)	Muscle (%)[#]	Bone (%)[#]	Skin and Fat (%)[#]
Con	67.89 ± 0.34	67.44 ± 1.23	26.12 ± 22.70	6.44 ± 0.41
aP-DF-G	69.22 ± 0.32	71.34 ± 0.62	22.15 ± 0.37	6.51 ± 0.37
aP-DF-L	69.03 ± 2.87	67.62 ± 1.83	25.08 ± 1.47	7.30 ± 0.52
tP-DF-G	64.67 ± 1.93	66.98 ± 2.00	26.83 ± 1.61	6.18 ± 0.58
tP-DF-L	66.24 ± 0.56	64.04 ± 0.89	28.14 ± 0.81	7.82 ± 0.62
aP-DFS-G	69.32 ± 0.67	67.68 ± 1.43	25.49 ± 1.83	6.83 ± 0.52
aP-DFS-L	65.85 ± 2.73	65.74 ± 1.63	26.64 ± 1.48	7.62 ± 0.64
tP-DFS-G	66.28 ± 0.74	65.73 ± 1.35	27.49 ± 1.57	6.99 ± 0.40
tP-DFS-L	64.83 ± 1.98	69.02 ± 1.91	25.10 ± 2.08	5.88 ± 0.45
p-value	0.29	0.07	0.22	0.14

[#]Calculated as a percentage of the breast weight
Con: Control diet formulated for available phosphorus, using mono-dicalcium phosphate, mixed with the grains.
aP-DF-G: Formulated for available phosphorus using de-fluorinated PA, mixed with the grains.
aP-DF-L: Formulated for available phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
tP-DF-G: Formulated for total phosphorus using de-fluorinated PA, mixed with the grains.
tP-DF-L: Formulated for total phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
aP-DFS-G: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
aP-DFS-L: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.
tP-DFS-G: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
tP-DFS-L: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.

Carcass component yield (%) results show that the breast and thigh portion weights do not differ ($p>0.05$) between the diets (Table 5.3). However, the wing and drum portions differ significantly with tP-DFS-L being significantly heavier than the rest. The ANOVA for thigh % indicated no differences between treatments ($p= 0.061$). Contrary to the ANOVA, after performing the Bonferroni's *post hoc* (least square means) test, differences could be seen with diet tP-DFS-L again having the highest yield. Çimrin and Demirel (2008) found the breast, thigh and wing portion weights of the birds that received sufficient phosphorus to be

significantly heavier than those with insufficient P. Angel *et al.* (2006) also found the birds receiving sufficient P to have significantly greater femur and tibia bone weights as well as tibia mineral density. A percentage of the portion weight differences might therefore be attributed to greater bone densities. These results differ to findings within the current study, as one of the treatments with the low P levels, diet tP-DFS-L (aP= 0.26-0.30%), gave rise to the highest portion yields as mentioned earlier. There is no biological explanation for the differences in these results (from that reported in the literature). It may, however, be attributed to the cutting method used when portioning the carcass which allowed for variation between these three different studies. Tables 5.1-5.3 indicate that the adding of the PA to the feed during mixing to have no effect on the carcass characteristics.

Table 5.3 Mean (± standard error) cold carcass component yield of the breast, thigh, leg and wing from broilers reared until 35 days of age on feeds formulated and mixed in different manners using two different phosphoric acids.

Treatment	Breast %[#]	Thigh %[#]	Drum %[#]	Wing %[#]
Con	39.56 ± 0.97	28.59^{ab} ± 0.79	13.96^{cd} ± 0.23	15.21^{e} ± 0.85
aP-DF-G	41.33 ± 0.44	27.69^{abc} ± 0.74	13.98^{cd} ± 0.26	15.75^{cde} ± 0.40
aP-DF-L	40.22 ± 1.19	28.14^{ab} ± 0.47	14.45^{bcd} ± 0.27	16.67^{bcde} ± 1.06
tP-DF-G	39.55 ± 1.16	27.54^{abc} ± 1.13	14.36^{bcd} ± 0.56	18.06^{ab} ± 0.84
tP-DF-L	39.58 ± 0.60	26.97^{bc} ± 0.79	15.34^{ab} ± 0.42	17.45^{abcd} ± 0.67
aP-DFS-G	39.24 ± 0.98	28.45^{ab} ± 0.55	14.90^{abc} ± 0.36	17.52^{abc} ± 0.26
aP-DFS-L	40.51 ± 0.66	29.29^{a} ± 0.88	13.83^{cd} ± 0.36	15.42^{de} ± 0.74
tP-DFS-G	38.20 ± 1.56	25.81^{c} ± 0.62	13.59^{d} ± 0.27	17.97^{ab} ± 0.93
tP-DFS-L	38.81 ± 1.84	29.28^{a} ± 0.83	15.69^{a} ± 0.54	18.97^{a} ± 0.47
p-value	0.71	0.06	<0.01	<0.01

[#]calculated as a percentage of the carcass weight
[a,b] means within columns that have different superscripts differ significantly ($p \leq 0.05$)
Con: Control diet formulated for available phosphorus, using mono-dicalcium phosphate, mixed with the grains.
aP-DF-G: Formulated for available phosphorus using de-fluorinated PA, mixed with the grains.
aP-DF-L: Formulated for available phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
tP-DF-G: Formulated for total phosphorus using de-fluorinated PA, mixed with the grains.
tP-DF-L: Formulated for total phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
aP-DFS-G: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
aP-DFS-L: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.
tP-DFS-G: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
tP-DFS-L: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.

5.5.2 Physical measurements

5.5.2.1 Colour and pH

The influence of PA on initial pH (pH_i) and ultimate pH (pH_u) of the breast and thigh muscles shows no significant difference ($p>0.05$) between diets on all muscle pH recordings (Table 5.4).

Table 5.4 Mean (± standard error) initial and ultimate pH measurements (CIE-Lab) for the breast and thigh muscle recorded from broilers reared until day 35 of age on feeds formulated and mixed in two different manners using two different phosphoric acids.

Treatment	Breast		Thigh	
	pH_i	pH_u	pH_i	pH_u
Con	6.24 ± 0.08	6.05 ± 0.04	6.40 ± 0.04	6.18 ± 0.06
aP-DF-G	6.28 ± 0.06	6.13 ± 0.05	6.26 ± 0.07	6.30 ± 0.04
aP-DF-L	6.23 ± 0.09	6.05 ± 0.04	6.18 ± 0.09	6.20 ± 0.05
tP-DF-G	6.05 ± 0.08	6.17 ± 0.07	6.28 ± 0.05	6.36 ± 0.08
tP-DF-L	6.27 ± 0.09	5.95 ± 0.05	6.27 ± 0.04	6.16 ± 0.05
aP-DFS-G	6.13 ± 0.05	6.06 ± 0.04	6.39 ± 0.05	6.32 ± 0.04
aP-DFS-L	6.07 ± 0.08	6.12 ± 0.05	6.29 ± 0.07	6.30 ± 0.07
tP-DFS-G	6.12 ± 0.08	5.90 ± 0.15	6.35 ± 0.11	6.27 ± 0.05
tP-DFS-L	6.25 ± 0.08	6.00 ± 0.04	0.39 ± 0.02	6.27 ± 0.05
p-value	0.27	0.13	0.25	0.17

[a,b] means within columns that have different superscripts differ significantly ($p\leq0.05$)
Con: Control diet formulated for available phosphorus, using mono-dicalcium phosphate, mixed with the grains.
aP-DF-G: Formulated for available phosphorus using de-fluorinated PA, mixed with the grains.
aP-DF-L: Formulated for available phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
tP-DF-G: Formulated for total phosphorus using de-fluorinated PA, mixed with the grains.
tP-DF-L: Formulated for total phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
aP-DFS-G: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
aP-DFS-L: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.
tP-DFS-G: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
tP-DFS-L: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.

Meat colour can be measured either by instrumental analysis or human appraisal (Zhu *et al.*, 1999). Colour measurements by the human eye are highly subjective and the sensitivity of the visual method is not the same as that of the instrumental analysis (Brewer & McKeith, 1999; Zhu *et al.*, 1999). Therefore, colour differences illustrated by instrumental means are not

always noticed by the human eye. The treatment had an effect ($p \leq 0.05$) on breast colour CIE-lab L*, a* and b* measurements as well as the chroma values, however, they did not affect the breasts' hue angle (Table 5.5). The Hue angle indicates the meat's colour with respect to the colour spectrum (red, yellow, orange, green, blue or violet), with an angle scale starting at 0°, to which the colour red is assigned, and ending at 90° which is assigned to yellow (Ponsano *et al.*, 2004). No significant differences are evident in the treatment's hue angles, however, all angles were greater than 85° and therefore the meat is a near-yellow colour. Chroma is an attribute which illustrates the meat's colour intensity or saturation. The chroma scale ranges from 0 to 60, with higher values indicating a more saturated colour. All chroma values of the current study were found to be on lower end of this scale, which is representative of a less saturated or intense colour shown by the meat. As mentioned, differences ($p \leq 0.05$) in chroma values between the treatments were observed, however, these saturation differences may be difficult to observe by the naked eye. Breast meat from the birds fed the tP-DF-L treatment diet had the highest recorded mean L* and b* values as well as chroma values. The higher L* indicates a paler meat colour than that of other treatments. Treatment tP-DF-G had the highest mean a* values. The lowest mean L* values were recorded for the tP-DF-G diet indicating the breast meat to be the darkest, however; it did not differ significantly from treatments aP-DF-G aP-DFS-L and tP-DFS-L. The lowest mean a* and b* values were recorded on the breasts of the chickens receiving diet aP-DFS-L. Han *et al.* (2012) reported L*, a* and b* measurements of 47.34, 10.65 and 18.74, respectively for the breast meat of birds fed phosphorus deficient diets (aP= 0.13%). These values differ from the deficient phosphorus (aP= 0.26-0.30%) diets (tP-DF-G, tP-DF-L, tP-DFS-G and tP-DFS-L) found within the current study as the L*, a* and b* measurements for these vary between 49.25-52.88, 0.05-0.69 and 11.60-11.49, respectively. Totosaus *et al.* (2007) reported that normal values of a* and b* for chicken breasts are 1.4 and 10.3, respectively. In the current study, b* values are close to that considered normal, however, the a* values were lower than that considered normal, indicating a reduced redness of the meat. All the L* values fall within the range of normality for poultry.

Table 5.5 Mean (± standard error) colour measurements (CIE-Lab) for the breast muscle gathered from broilers reared until day 35 of age on feeds formulated and mixed in two different manners using two different phosphoric acids.

Treatment	Chroma	Hue	Breast		
			L*	a*	b*
Con	10.58^{ab}± 0.32	85.09 ± 0.76	50.53^{bc} ± 0.64	0.51^{ab} ± 0.21	10.53^{bcde} ± 0.32
aP-DF-G	10.01^{ab}± 0.26	85.42 ± 0.66	51.48^{ab} ± 0.65	-0.17^{cd} ± 0.20	9.97^{de} ± 0.27
aP-DF-L	10.99^{ab}± 0.37	86.35 ± 0.87	50.54^{bc} ± 0.42	0.54^{ab} ± 0.23	10.95^{abcd} ± 0.35
tP-DF-G	11.12^{ab}± 0.39	85.44 ± 0.91	49.25^{c} ± 0.59	0.69^{a} ± 0.24	11.06^{abc} ± 0.39
tP-DF-L	11.53^{a} ± 0.20	86.40 ± 0.70	52.88^{a} ± 0.83	0.05^{bc} ± 0.22	11.49^{a} ± 0.20
aP-DFS-G	10.85^{ab}± 0.41	85.59 ± 0.84	50.53^{bc} ± 0.53	0.25^{abc} ± 0.21	10.09^{cde} ± 0.41
aP-DFS-L	9.85^{b} ± 0.35	85.03 ± 0.50	51.62^{ab} ± 0.73	-0.58^{d} ± 0.16	9.81^{e} ± 0.36
tP-DFS-G	11.38^{ab}± 0.45	86.16 ± 0.70	51.04^{b} ± 0.75	0.21^{abc} ± 0.21	11.34^{ab} ± 0.46
tP-DFS-L	11.22^{ab}± 0.38	86.72 ± 0.62	52.17^{ab} ± 0.52	0.19^{abc} ± 0.21	11.19^{ab} ± 0.38
p-value	0.69	<0.01	<0.01	<0.01	<0.01

^{a,b} means within columns that have different superscripts differ significantly ($p \leq 0.05$)
Con: Control diet formulated for available phosphorus, using mono-dicalcium phosphate, mixed with the grains.
aP-DF-G: Formulated for available phosphorus using de-fluorinated PA, mixed with the grains.
aP-DF-L: Formulated for available phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
tP-DF-G: Formulated for total phosphorus using de-fluorinated PA, mixed with the grains.
tP-DF-L: Formulated for total phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
aP-DFS-G: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
aP-DFS-L: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.
tP-DFS-G: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
tP-DFS-L: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.

A number of studies have been conducted in an attempt to acquire pH and CIE-Lab measurements of broiler meat which gives rise to a normal colour that is pleasing to the consumer (Fletcher, 1999; Van Laack *et al.*, 2000; Qiao *et al.*, 2001). There is a strong correlation between meat colour and pH, specifically that lighter meat with higher L* values generally shows lower pH values and *vice versa* for dark meat (Mancini & Hunt, 2005). Pale meat with high L* values are indicative of poor meat quality (Chen *et al.*, 2013). This being said, there is much variation in published studies regarding normal colour meat's L* values in chickens. Fletcher (1999) reported a value of 45.6, Van Laack *et al.* (2000) reported 55.1 and Qiao *et al.* (2001) reported values between 48 and 53 for normal meat. Van Laack *et al.* (2000) also reported ultimate pH values for normal meat to be 5.96 and 5.70 for pale meat whilst Qiao *et al.* (2001) reported dark meat to have a pH of 6.23 and higher. Therefore, the results in the current study show the meat to be normal in colour for all treatments, as the L* values fall between the extremes of that reported by Qiao *et al.* (2001). Furthermore, all breast pHi and pHu values are greater than that which Van Laack *et al.* (2000) reported as indicative of pale, soft and exudative meat. A number of the thigh pH_u values are above 6.23 and so may be considered as dark meat; the reasons for these higher pH values are not clear, as the *ante-mortem* treatments were similar for all birds. No clear trends in the data illustrate that adding the PA to the grains or adding it last in the feed mixing process have an effect on breast colour and pH and thigh pH.

5.6 Conclusion

The present study assessed the effects of two different phosphoric acids and their application methods on the carcass characteristics and meat colour and pH of broilers grown under standard commercial conditions. Live weight, as well as cold and warm carcass weight was not affected by the source of phosphorus, provided that the diets were formulated for the correct amount of available phosphorus. The order of mixing the PA into the diet had no effect on any of the measurements taken in the study. Overall, the two phosphoric acids did not influence breast and thigh pH. Differences were observed in the breast muscle CIE-Lab measurements (L*, a* and b*) and chroma values. However, the conclusion was made that neither pH nor diet differences give cause for the variation seen in the breast colour, therefore further studies will be needed to assess this variation. The conclusion was made that defluorinated PA and

defluorinated and desulfonated PA can be used as a source of phosphorus in broiler diets with no adverse negative effects on the carcass characteristics or quality of the meat.

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Chapter 6

The effects of phosphoric acid on organ and gut measurements and bone parameters of broiler chickens

6.1 Abstract

The purpose of this study was to evaluate the effects of defluorinated phosphoric acid (DF) and defluorinated and desulfonated phosphoric acid (DFS) on organ weight, intestinal pH, gizzard erosion scores and bone breakage as well as bone mineralisation of broiler chickens. The following nine treatment diets were used: 1. The control (Con); 2. Formulated for available phosphorus (aP) using DF and mixed with the grains of the diet (G) (aP-DF-G); 3. Formulated for aP using DF, mixed in at the end of the mixing process (L) (aP-DF-L); 4. Formulated for total phosphorus (tP) using DF, that was mixed with the grains (tP-DF-G); 5. Formulated for tP using DF, mixed in at the end of the mixing process (tP-DF-L) 6. Formulated for aP using DFS, that was mixed with the grains (aP-DFS-G); 7. Formulated for aP using DFS, mixed in at the end of the mixing process (aP-DFS-L); 8. Formulated for tP using DFS, that was mixed with the grains (tP-DFS-G); 9. Formulated for tP using DFS, mixed in at the end of the mixing process (tP-DFS-L). No treatment differences were found for heart and bursa of Fabricius weights as well as weights of the heart, liver, gizzard, spleen and bursa of Fabricius relative to body weight as well as the spleen to bursa ratio ($p>0.05$). Differences, however, were found in the gizzard, spleen and liver weights ($p\leq0.05$). No significant difference was found in the proventriculus, duodenum, jejunum and ileum but differences can be seen in the cecum ($p= 0.014$). Significant differences are evident in the bone strength, the diets formulated for aP being significantly stronger than those formulated for tP.

6.2 Introduction

The major gut functions of digestion, absorption and intestinal barrier are very important in monogastric nutrition. Fast and efficient production rely heavily on these processes and optimization should be performed with minimal nutrient use (Van der Klis & Jansman, 2002). Not only is the digestive tract essential for digestion and absorption, but it is also the largest immunological organ as it is the first site of protection against pathogens (Choct, 2009). For efficient nutrient digestion and absorption, one needs to ensure that the health and integrity of

the gastro-intestine are maintained. Young broilers need to develop their gastrointestinal tract (GIT) quickly to keep up with the birds' nutritional demands (Uni *et al.*, 1996; Iji *et al.*, 2001). Therefore, the bird's GIT has to develop concurrently with the bird (Uni *et al.*, 1998). However, digestion may be highly compromised during the first eight days due to lower enzyme activity (Nitson *et al.*, 1991), specifically when they are fed diets with anti-nutritional factors. An example of this is the myoinositol hexaphosphate (IP6) compound. This is found in grains bound to phosphorus and is known as phytate. Much has been reported on the anti-nutritional factors of phytate (Gillis *et al.*, 1954; Ammerman *et al.*, 1961; Hurwitz & Bar, 1965; De Groote & Huyghebaert, 1997; Kornegay, 2000; Angel, 2010; Kleyn, 2013; Shastak & Rodehutscord, 2013).

The pH of the digesta is one of the most influential factors of nutrient bioavailability (Pang & Applegate, 2007). It is of utmost importance that the GIT pH is maintained constant at the optimal level, as even small changes can significantly affect mineral digestion and absorption (Bristol, 2003). Therefore, digesta pH readings could be used as an indication of gut health and nutrient absorption (Bristol, 2003). Furthermore, pH is important to keep animals healthy by maintaining a good balance of pathogenic and non-pathogenic microorganisms (Pang & Applegate, 2007). This has been seen in the case of neonatal rabbits where gastric acidity protected the rabbits against translocation of potentially pathogenic bacteria (Dinsmore *et al.*, 1997).

A bird's immune status is affected by nutrient deficiencies (Kwak *et al.*, 1999). This is illustrated by the findings of Kwak *et al.* (1999), where low levels of arginine caused the lymphoid organs to develop poorly. Two lymphoid organs are of significance to the current study, namely the bursa of Fabricius and spleen, of which both are important to the immune system (Yegani & Korver, 2008). Lymphoid organs resist host invasion by pathogens, fight against infections and most importantly, ensure productivity is not affected during an infectious attack (Kwak *et al.*, 1999). Feed has the ability to alter organs' structural integrity through feed granule size (Engberg *et al.*, 2002), as well as the nutritional composition (Fasina *et al.*, 2006). Therefore, knowledge of the nutritional quality of feed is essential for good growth and development of the organs and the bird as a whole (Ensminger, 1992).

The skeleton (bone) is a dynamic tissue which can be influenced by nutritional, physiological and physical factors (Rath *et al.*, 2000). Over the years, genetic selection for rapidly growing

broilers has resulted in greater instances of bone development problems and loss of structural bone integrity, otherwise referred to as skeletal disorders (Williams *et al.*, 2000). Skeletal disorders are some of the most common problems found in poultry production (Kestin *et al.*, 1992). They not only affect the birds' welfare status, but can reduce growth and be the cause of higher mortality rates. as well as increase the occurrence of carcass downgrading (Williams *et al.*, 2000). Furthermore, inadequate levels of calcium and phosphorus within the diet may lead to bone fractures and deformities (Driver, 2004). Bone status can be an indicator of the mineral adequacy of the diet and the degree of bone mineralisation affects the strength of the bone. Bone ash has been used to determine the quantity of phosphorus deposition in the bone and bone breaking force as an indicator of bone strength (Shastak, 2012).

Phosphorus clearly plays an important role in skeletal development and maintenance (Soares, 1995), pH and maintenance of the digestive tract (Miles & Henry, 1997). Therefore objectives of this study were:

i. To investigate the effects of a defluorinated phosphoric acid and a defluorinated and desulfonated phosphoric acid as the phosphorus source on organ weights, gizzard scores and intestinal pH.
ii. To investigate the effects of the phosphoric acids on tibia phosphorus and calcium content as well as to determine their effects on tibia bone strength.
iii. To evaluate the effects of adding the phosphoric acids to the diet at two inclusion levels on parameters mentioned in objectives i and ii.
iv. To determine the effects of adding the phosphoric acid to the diet at different intervals during the mixing process on parameters mentioned in objectives i and ii.

6.3 Materials and methods

Extensive details on experimental layout, diets, animals and the housing are given in Chapter 4. To provide a concise description, nine treatments were allocated evenly and randomly to fifty-four cages with ten replicates per cage. The treatment diets are as follows:

1. Con: Control diet formulated for available phosphorus, using a mono-dicalcium phosphate, which was mixed with the grains.
2. aP-DF-G: Formulated for available phosphorus using a de-fluorinated PA, that was mixed with the grains.

3. aP-DF-L: Formulated for available phosphorus using de-fluorinated PA, mixed in at the end of the mixing process.
4. tP-DF-G: Formulated for total phosphorus using de-fluorinated PA, which was mixed with the grains.
5. tP-DF-L: Formulated for total phosphorus using de-fluorinated PA, mixed in at the end of the mixing process.
6. aP-DFS-G: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, which was mixed with the grains.
7. aP-DFS-L: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed in at the end of the mixing process.
8. tP-DFS-G: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, which was mixed with the grains.
9. tP-DFS-L: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed in at the end of the mixing process.

The diets were fed *ad libitum* until slaughter at 33 days of age. On day 33, one bird per pen was randomly selected from near the pens' mean weight and slaughtered according to standard commercial practice.

6.3.1 Bone breakage strength

Both tibias were removed post slaughter and frozen at -20ºC until further analysis. The right tibia was thawed and cleaned of any adherent tissue of any sort before the weight was recorded. Thereafter, breaking strength was determined by a three point bending test as described by Fleming *et al.*, (1998) using an Instron® tensile machine with a maximum force of 2 kilo-newton (kN). This method is in accordance with that prescribed by Fleming *et al.* (1998). The centre of the bone was marked and placed between two 10 mm retaining bars set 40 mm apart. A 10 mm diameter crosshead probe approached the anterior of the tibia at a speed of 30 mm/min. The maximum load, before failure occurred, was recorded as the breaking strength (N). Breaking force relative to bone weight was calculated using Equation 6.1.

Equation 6.1

$$\text{Breaking force (N/g)} = \frac{\text{Force (N)}}{\text{Bone weight (g)}}$$

6.3.2 Bone mineral content

The left tibia bone was thawed and cleaned of any adherent tissue. Official method 934.01 of the AOAC (2002) was used to determine dry matter of the bone. The bones were placed in a dry porcelain crucible and dried for 24 hours at 100ºC. Thereafter, the bones were placed in a desiccator for 30 minutes and subsequently weighed. Defatting then took place by submerging the bones in petroleum ether for 48 hours after breaking the bone in half to facilitate defatting (Rama Rao & Ramasubba Reddy, 2001). The bones were then dried at 100ºC for 24 hours to obtain fat free dry bone weight. Subsequently, the fat free bones were placed in a furnace at 600ºC for 24 hours to obtain fat-free bone ash percentage. All bone weights were measured using a Mettler AE 200 scale (Mettler-Toledo, Switzerland) with 0.0001g accuracy.

Mineral analysis of the ashed bone samples were performed at the Western Cape Department of Agriculture in Elsenburg according to the combustion method No. 6.1.1 in ALASA (2007). An in-depth description of the procedure is given in section 3.3.4.8.

6.3.3 Organ weights and gizzard score

All intestinal pH recordings and organ weights were taken at Mariendahl Experimental farm (Stellenbosch University, Western Cape). Five organs, the heart, gizzard, spleen, liver and bursa of Fabricius, were removed from the fresh carcass immediately after slaughter and weighted using a Mettler PC 4400 laboratory scale (Mettler-Tolado, Switzerland). A longitudinal cut through the gizzard allowed it to be opened and cleaned of any remaining feed. After cleaning and weighing, the gizzard was scored for gizzard erosion using an ordinal scale shown in Table 6.1.

Table 6.1 Gizzard erosion scoring scale.

Score	Description
0	No erosion
1	Slight erosion (rough epithelia)
2	Modest erosion (rough and distinct gaps)
3	Severe erosion (rough, gaps and ulcers showing on the stomach wall)
4	Extreme erosion (rough, gaps, ulcers and separation of the epithelia from the stomach wall)

6.3.4 pH measurements

After organ removal, pH measurements were taken from the proventriculus, duodenum, jejunum (near the centre), ileum and cecum using a calibrated (buffers pH 4.0 and 7.0 at room temperature) Crison pH25 meter (Alella, Barcelona). The electrode was inserted into the middle of the area of the intestine to be measured and between every reading the probe was rinsed with distilled water.

6.4 Statistical analysis

Data from the trial were analysed using STATISTICA (Dell Inc., 2016). The assumptions of homoscedasticity and normality were investigated prior to any further analyses. The test significance was set at $p \leq 0.05$. All treatment effects were analysed using one-way (ANOVA) analysis of variance and Bonferroni's *post hoc* (least square means) test for the significant data, except gizzard erosion scores. Gizzard erosion scores were analysed using the chi-squared test of STATISTICA.

6.5 Results and discussion

6.5.1 Bone Breakage Strength

The results of bone strength are shown in Table 6.2. The breaking strength results are expressed as the force required to break the bone in Newton (N) and as the breaking force required per gram of bone (N/g). Differences were evident between treatments within both parameters ($p \leq 0.05$). A significant increase in breaking strength (N) and breaking strength per gram of bone (N/g) was distinctive between treatments formulated for total (tP) and available phosphorus (aP) where treatments formulated for aP had greater values throughout indicating stronger bones. Formulating for aP and tP has an effect on the inclusion levels of the P source, with those diets formulated according to aP receiving higher P inclusion levels than that made according to tP. Venäläinen *et al.* (2006) tested the effects of different levels of aP (3.5, 4.0, 4.5 and 5.0 g/kg aP) on tibia bone breakage. Results of the study showed no differences in bone breakage between the different levels of aP. This was not the case with the study at hand. The current study recorded aP values for the diets formulated for tP and aP to be 0.26-0.30% and 0.42-0.50%, respectively, and the results, with reference to P inclusion levels on bone breakage, are in agreement with those obtained by Shaw *et al.* (2010) and Aureli *et al.* (2011). Shaw *et*

al. (2010) tested three phytase enzymes on male broiler chicks with one treatment having low amounts of available phosphorus (aP) (aP= 0.25%). They found the diet with low aP to cause a substantially lower tibia breaking force to that with adequate aP (aP= 0.45%). Aureli *et al.* (2011) also had a negative control with aP of 0.30%, yielding similar results to that of Shaw *et al.* (2010). However, these studies are not entirely comparable to the current study as they test the effects of phytases. Nevertheless, their results illustrate the negative effects insufficient aP in the diet has on tibia bone strength.

There is little clear information indicating a difference in tibia strength with reference to the PA used in the diet. The same can be said for the method of mixing the PA in with the feed. The conclusion can therefore be made that the defluorinated and the defluorinated and desulfonated phosphoric acid are sufficient in maintaining bone strength when used as the dietary phosphorus source, provided the inclusion levels are adequate for maintenance.

Table 6.2 Mean (± standard error) tibia strength obtained from broilers reared until 33 days of age on feeds formulated and mixed in different manners using two different phosphoric acids.

Treatment	Tibia strength (N)	Tibia strength (N/g)
Con	348.58^{a} ± 33.61	29.00^{a} ± 0.56
aP-DF-G	328.00^{ab} ± 16.55	29.51^{a} ± 0.74
aP-DF-L	375.90^{a} ± 11.65	31.16^{a} ± 0.8
tP-DF-G	258.35^{cd} ± 37.40	22.42^{ab} ± 2.67
tP-DF-L	237.05^{d} ± 27.69	21.25^{b} ± 1.22
aP-DFS-G	372.52^{a} ± 18.31	29.72^{a} ± 0.97
aP-DFS-L	309.00^{abc} ± 6.01	28.20^{a} ± 0.82
tP-DFS-G	261.92^{bcd} ± 25.11	22.98^{ab} ± 2.13
tP-DFS-L	214.52^{d} ± 19.09	19.19^{b} ± 1.31
p-value	<0.01	<0.01

^{a,b} means within columns that have different superscripts differ significantly ($p \leq 0.05$)
Con: Control diet formulated for available phosphorus, using mono-dicalcium phosphate, mixed with the grains.
aP-DF-G: Formulated for available phosphorus using de-fluorinated PA, mixed with the grains.
aP-DF-L: Formulated for available phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
tP-DF-G: Formulated for total phosphorus using de-fluorinated PA, mixed with the grains.
tP-DF-L: Formulated for total phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
aP-DFS-G: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
aP-DFS-L: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.
tP-DFS-G: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
tP-DFS-L: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed last in at the end of the mixing process.

6.5.2 Mineralisation

No significant differences in tibia Ca and P contents were found between dietary treatments (Table 6.3). Earlier studies have reported an increase in bone mineralisation with the increase in dietary aP (Nelson *et al.*, 1990; Onyango *et al.*, 2003; Venäläinen *et al.*, 2006). Venäläinen *et al.* (2006) tested the effects of tibia ash, Ca and P content between broilers fed diets with increasing levels of dietary aP (3.5, 4.0, 4.5 and 5.0 g/kg aP). The authors found the levels of Ca and P to increase curvilinearly as the levels of dietary aP increase. Simmilar to the studies of Nelson *et al.* (1990), Onyango *et al.* (2003) and Venäläinen *et al.* (2006), the current study had different levels of aP between the diets (aP= 2.6-3.0 and 4.2-5.0g/kg). However, the results differ from that reported in the previous studies.

The bones status is a common tool used to indicate a diet's mineral adequacy. It is also well known that Ca and P are major minerals responsible for bone formation (Soares, 1995; Mc Donald *et al.*, 2011). Therefore, any deficency or inadequacy of these minerals should result in lower mineral levels in the bone. The reasons for this not occuring in the current study cannot be explained and therefore further research must be persued in order to obtain the possible answers.

Table 6.3 Mean (± standard error) calcium and phosphorus content (as a % of bone ash) of a fat free tibia bone from broilers reared until 33 days of age on feeds formulated and mixed in different manners using two different phosphoric acids.

Treatment	Calcium	Phosphorus	Ca:P
Con	69.80 ± 17.99	21.65 ± 3.19	3.36 ± 1.32
aP-DF-G	63.12 ± 15.25	22.95 ± 4.72	2.96 ± 1.31
aP-DF-L	63.36 ± 14.38	22.29 ± 3.15	2.92 ± 0.84
tP-DF-G	65.36 ± 10.74	25.66 ± 4.28	2.66 ± 0.87
tP-DF-L	59.13 ± 11.31	21.90 ± 4.69	2.83 ± 0.90
aP-DFS-G	53.99 ± 12.01	24.40 ± 3.52	2.26 ± 0.60
aP-DFS-L	67.60 ± 17.53	23.74 ± 2.32	2.89 ± 0.88
tP-DFS-G	67.70 ± 13.47	23.59 ± 2.25	2.86 ± 0.46
tP-DFS-L	57.15 ± 10.72	22.12 ± 2.24	2.59 ± 0.64
p-value	0.55	0.58	0.74

Con: Control diet formulated for available phosphorus, using mono-dicalcium phosphate, mixed with the grains.
aP-DF-G: Formulated for available phosphorus using de-fluorinated PA, mixed with the grains.
aP-DF-L: Formulated for available phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
tP-DF-G: Formulated for total phosphorus using de-fluorinated PA, mixed with the grains.
tP-DF-L: Formulated for total phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
aP-DFS-G: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
aP-DFS-L: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.
tP-DFS-G: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
tP-DFS-L: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed last in at the end of the mixing process.

6.5.3 Gizzard score

The scoring of gizzard erosion is the visual analysis of the gizzard for lesions or blood spots within the gizzard lining as a result of the dietary treatments, once it has been cut open and cleaned of any remaining feed. A number of factors have been identified which lead to gizzard erosion taking place. These factors are feed structure, nutritional deficiencies, infections,

mycotoxins, microbial colonisation and congenital factors (Gjevre *et al.*, 2013). Results found within the current study are reported in Table 6.4. In the present study, very few severe cases of gizzard erosion were present. Therefore, phosphoric acid, be it defluorinated or defluorinated and desulfonated, had no adverse effects on the gizzard no matter the inclusion levels of the PA nor the procedure used during the mixing of the feed.

Table 6.4 Mean (± standard error) gizzard erosion obtained from broilers reared until 33 days of age on feeds formulated and mixed in different manners using two different phosphoric acids.

Treatment	Gizzard score				
	0	**1**	**2**	**3**	**4**
Con	0	2	2	2	0
aP-DF-G	0	1	2	2	1
aP-DF-L	0	0	3	3	0
tP-DF-G	0	0	4	2	0
tP-DF-L	0	0	3	3	0
aP-DFS-G	0	1	2	3	0
aP-DFS-L	0	3	3	0	0
tP-DFS-G	0	2	3	1	0
tP-DFS-L	0	1	3	2	0
Chi-square p-value	0.60				

Con: Control diet formulated for available phosphorus, using mono-dicalcium phosphate, mixed with the grains.
aP-DF-G: Formulated for available phosphorus using de-fluorinated PA, mixed with the grains.
aP-DF-L: Formulated for available phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
tP-DF-G: Formulated for total phosphorus using de-fluorinated PA, mixed with the grains.
tP-DF-L: Formulated for total phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
aP-DFS-G: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
aP-DFS-L: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.
tP-DFS-G: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
tP-DFS-L: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed last in at the end of the mixing process.

6.5.4 Organ weights

The results of organ weight (g) and organ weight relative to body weight (%), together with spleen to bursa ratio, are shown in Tables 6.5 and 6.6 respectively. No significant differences

were recorded in the heart and the bursa weighs between the treatments. Differences ($p \leq 0.05$) were observed in the liver, spleen and gizzard. However, these differences were alleviated ($p > 0.05$) when expressed as a percentage of live weight, and no differences were found in the spleen to bursa ratio ($p > 0.05$). The broilers fed a diet formulated for aP with a defluorinated phosphoric acid as the phosphorus source which had been mixed into the grain content of the diet, were found to have the highest organ weights, not only for those which were found to be significantly different between dietary treatment but for all the organ weights recorded. This might be a result of the birds being heavier in weight, however, when expressed relative to body weight the differences were alleviated and so this cannot be the case. Further research will be needed to pin point the cause of heavier organ weights seen in this treatment.

When assessing the immune status of a chicken, the measurement of the lymphoid organ weights is known to be an accurate tool off assessment (Heckert *et al.*, 2002). The increase in lymphoid organ weight may indicate an improved immune system (Nourmohammadi *et al.*, 2011). However, one must be aware of the implications that an excessive immune response might have on the performance of the bird (Collett *et al.*, 2005). A reduced bursa weight is indicative of stress or possible viral infection (Pope, 1991).

Table 6.5 Mean (± standard error) organ weights obtained from broilers reared until 33 days of age on feeds formulated and mixed in different manners using two different phosphoric acids.

Treatment	Heart (g)	Liver (g)	Gizzard (g)	Spleen (g)	Bursa (g)
Con	10.13 ± 1.16	35.25^{b} ± 5.04	29.09^{abc} ± 4.68	1.59^{bc} ± 0.28	4.33 ± 1.37
aP-DF-G	13.13 ± 2.78	41.68^{a} ± 6.71	32.60^{a} ± 4.65	2.12^{a} ± 0.61	5.41 ± 1.11
aP-DF-L	11.67 ± 1.31	34.52^{b} ± 1.73	31.19^{ab} ± 3.04	1.95^{ab} ± 0.35	3.38 ± 1.24
tP-DF-G	11.24 ± 1.61	30.87^{bc} ± 6.72	23.13^{d} ± 3.57	1.41^{cd} ± 0.42	3.34 ± 1.06
tP-DF-L	12.37 ± 2.34	32.18^{bc} ± 3.46	26.21^{bcd} ± 6.66	1.44^{cd} ± 0.28	3.82 ± 2.25
aP-DFS-G	12.17 ± 2.74	36.01^{ab} ± 4.60	32.40^{a} ± 7.31	1.71^{abc} ± 0.37	4.14 ± 0.98
aP-DFS-L	11.94 ± 2.16	33.90^{b} ± 3.44	28.37^{abcd} ± 4.61	1.66^{bc} ± 0.41	4.40 ± 2.00
tP-DFS-G	10.32 ± 2.32	27.92^{c} ± 6.46	23.01^{d} ± 4.74	1.07^{d} ± 0.37	3.34 ± 0.78
tP-DFS-L	10.33 ± 1.66	31.33^{bc} ± 4.45	24.21^{cd} ± 3.16	1.43^{cd} ± 0.39	3.17 ± 1.23
p-value	0.19	<0.01	<0.01	<0.01	0.15

^{a,b} means within columns that have different superscripts differ significantly ($p \leq 0.05$)
Con: Control diet formulated for available phosphorus, using mono-dicalcium phosphate, mixed with the grains.
aP-DF-G: Formulated for available phosphorus using de-fluorinated PA, mixed with the grains.
aP-DF-L: Formulated for available phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
tP-DF-G: Formulated for total phosphorus using de-fluorinated PA, mixed with the grains.
tP-DF-L: Formulated for total phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
aP-DFS-G: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
aP-DFS-L: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.
tP-DFS-G: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
tP-DFS-L: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed last in at the end of the mixing process.

Table 6.6 Mean (± standard error) organ weights relative to body weight and the spleen to bursa ratio obtained from broilers reared until 33 days of age on feeds formulated and mixed in different manners using two different phosphoric acids.

Treatment	Heart (%)	Liver (%)	Gizzard (%)	Spleen (%)	Bursa (%)	Spleen:Bursa
Con	0.51 ± 0.10	1.77 ± 0.22	1.48 ± 0.33	0.09 ± 0.01	0.22 ± 0.08	0.39 ± 0.11
aP-DF-G	0.57 ± 0.14	1.81 ± 0.36	1.41 ± 0.21	0.09 ± 0.04	0.23 ± 0.05	0.42 ± 0.19
aP-DF-L	0.56 ± 0.05	1.67 ± 0.17	1.50 ± 0.11	0.09 ± 0.01	0.16 ± 0.06	0.62 ± 0.15
tP-DF-G	0.72 ± 0.23	1.73 ± 0.26	1.46 ± 0.30	0.08 ± 0.02	0.21 ± 0.05	0.43 ± 0.11
tP-DF-L	0.68 ± 0.16	1.76 ± 0.20	1.43 ± 0.35	0.08 ± 0.02	0.21 ± 0.12	0.47 ± 0.22
aP-DFS-G	0.59 ± 0.11	1.78 ± 0.21	1.58 ± 0.27	0.08 ± 0.02	0.20 ± 0.03	0.43 ± 0.13
aP-DFS-L	0.55 ± 0.12	1.60 ± 0.18	1.35 ± 0.28	0.08 ± 0.02	0.21 ± 0.11	0.40 ± 0.08
tP-DFS-G	0.62 ± 0.18	1.65 ± 0.37	1.35 ± 0.24	0.06 ± 0.02	0.20 ± 0.04	0.32 ± 0.10
tP-DFS-L	0.68 ± 0.13	2.05 ± 0.37	1.58 ± 0.26	0.09 ± 0.03	0.21 ± 0.09	0.47 ± 0.08
p-value	0.22	0.26	0.75	0.18	0.92	0.07

Con: Control diet formulated for available phosphorus, using mono-dicalcium phosphate, mixed with the grains.
aP-DF-G: Formulated for available phosphorus using de-fluorinated PA, mixed with the grains.
aP-DF-L: Formulated for available phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
tP-DF-G: Formulated for total phosphorus using de-fluorinated PA, mixed with the grains.
tP-DF-L: Formulated for total phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
aP-DFS-G: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
aP-DFS-L: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.
tP-DFS-G: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
tP-DFS-L: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed last in at the end of the mixing process.

6.5.5 Intestinal pH

The gut environmental conditions play a major role in nutrient digestion and absorption (Rahmani *et al.*, 2005). The internal surface of the small intestine (SI) is surrounded by luminal fluid. This fluid maintains the micro environment in which the digestion and absorption of nutrients take place (Mitchell & Lemme, 2008). The pH of the gastrointestinal tract (GIT) is affected by a number of factors, such as the kind of nutrients in the intestine, chicken health as a whole and the microflora of the intestines. Furthermore, the pH in different sections of the GIT affects the digestion and absorption of nutrients there (Rahmani *et al.*, 2005).

No significant differences in the pH values of the proventriculus, duodenum, jejunum and ileum were found between the dietary treatments of the current study (Table 6.7). Differences were, however, seen in the cecum ($p= 0.014$). As expected, the pH of the luminal fluid increased from the proximal to the distal area of the SI. However, all values do portray a higher acidity, as the pH of the SI is generally found to lie between 6.5 and 7.5 (Simon & Igbasan, 2002). Van der Klis & Jansman (2002) reported normal pH values specific to the duodenum, jejenum and ileum to range between 5.5-6.2, 5.8-6.9 and 6.3-8.0, respectively. The current study's values are on the acidic extremes of both of these studies. This higher acidity in the SI is advantageous as it has been reported to increase nutrient absorption (Rahmani *et al.*, 2005); yet Rayssiguier & Remesy (1977) reported a decrease in cecal pH as a result of increased microbial fermentation through higher substrate levels within the cecum of rats. This means a larger concentration of nutrients were not absorbed in the SI sections preceding the cecum. Perhaps the fact that a PA was used could explain these lower pH values. However, the control diet uses a commercial MDCP and had similar pH values to those diets using the PA throughout. Therefore, further research will be required to explain these results.

Table 6.7 Mean (± standard error) pH of various areas of the digestive tract obtained from broilers reared until 33 days of age on feeds formulated and mixed in different manners using two different phosphoric acids.

Treatment	Proventriculus	Duodenum	Jejunum	Ileum	Cecum
Con	2.63 ± 0.97	5.52 ± 0.55	5.76 ± 0.24	5.99 ± 0.30	5.83^{bc} ± 0.17
aP-DF-G	2.57 ± 0.89	5.52 ± 0.31	5.72 ± 0.24	5.73 ± 0.32	5.68^{c} ± 0.41
aP-DF-L	2.88 ± 0.55	5.07 ± 0.75	5.73 ± 0.78	6.01 ± 0.53	6.04^{bc} ± 0.33
tP-DF-G	2.66 ± 0.87	5.36 ± 0.47	5.74 ± 0.14	5.67 ± 0.22	5.76^{bc} ± 0.49
tP-DF-L	3.27 ± 0.93	5.20 ± 0.47	5.75 ± 0.21	5.81 ± 0.23	5.71^{bc} ± 0.39
aP-DFS-G	2.96 ± 0.70	5.69 ± 0.24	5.77 ± 0.10	6.07 ± 0.28	6.14^{ab} ± 0.16
aP-DFS-L	3.13 ± 1.52	5.74 ± 0.27	5.75 ± 0.20	5.98 ± 0.51	6.51^{a} ± 0.38
tP-DFS-G	3.51 ± 1.04	5.51 ± 0.34	5.74 ± 0.27	5.52 ± 0.25	5.88^{bc} ± 0.63
tP-DFS-L	3.36 ± 1.14	5.69 ± 0.19	5.79 ± 0.19	5.81 ± 0.20	6.14^{abc} ± 0.31
p-value	0.68	0.14	1.00	1.78	0.01

^{a,b} means within columns that have different superscripts differ significantly ($p \leq 0.05$)
Con: Control diet formulated for available phosphorus, using mono-dicalcium phosphate, mixed with the grains.
aP-DF-G: Formulated for available phosphorus using de-fluorinated PA, mixed with the grains.
aP-DF-L: Formulated for available phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
tP-DF-G: Formulated for total phosphorus using de-fluorinated PA, mixed with the grains.
tP-DF-L: Formulated for total phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
aP-DFS-G: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
aP-DFS-L: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.
tP-DFS-G: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
tP-DFS-L: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed last in at the end of the mixing process.

6.6 Conclusion

The aim of the present research was to examine the effects of a defluorinated and a defluorinated and desulfonated phosphoric acid (PA), which were mixed into the diet in two different procedures and at two inclusion levels, tibia breaking strength and tibia phosphorus and calcium content, gizzard scores, organ weights and intestinal pH.

Supplementing broiler diets with PA shows no improvement nor deterioration in tibia breaking strength provided the inclusion levels of PA were sufficient. Those fed a diet with sufficient P levels had significantly higher breaking strength than those fed diets deficient in P. Only organ weighs of the heart and bursa of Fabricius were unaffected by the treatment differences. Liver,

gizzard and spleen weights were affected by the dietary treatments; however, after expressing the organ weights relative to whole body weight, no differences were evident. Alteration in the spleen to bursa ratio was not found. Therefore, it is concluded that PA had no effect on the immune system. Gizzard score, tibia Ca and P content and pH recordings of the proventriculus, duodenum, jejunum and ileum were unaffected by the differences in dietary treatments. Differences were found in the pH recordings of the cecum. These intestinal pH recordings were seen to be lower than that typically found in literature and this may be a result of the PA.

6.7 References

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Chapter 7

General conclusion

The primary objective of the first experiment within this study was to evaluate the phosphorus (P) bioavailability of a defluorinated phosphoric acid (DF) when supplemented into broiler diets. The secondary objective was to determine the effects of this phosphoric acid (PA) on nutrient and mineral digestibility. Both objectives were completed by means of a digestibility study. Phosphorus bioavailability results showed the dilution diet (100% DF) to have higher mean P bioavailability than the other diets. The depreciation in P bioavailability from the treatments that had the summit and dilution diet mixed together was taken to be a cause of interaction between the two phosphate sources. The dilution diet also resulted in greater coefficient of total tract digestibility (CTTD) values of protein, P, calcium, magnesium, potassium, iron, sodium, copper, zinc, manganese, boron and aluminium. A higher digestibility of protein gives rise to a better amino acid balance within the diet which is advantageous to the bird. The knowledge of the P bioavailability value and the higher P bioavailability of defluorinated PA gives the opportunity for nutritionists to formulate diets closer to the birds' requirements. This leads to less P excretion and therefore the negative effects on the environment can be reduced. Furthermore, as inorganic phosphates are very expensive, formulating a diet which better meets requirements results in less wastage and ultimately reduced feed costs.

As pertaining to the second experiment, not only was a defluorinated PA utilized but also defluorinated and desulfonated (DFS) phosphoric acid. The dietary treatment differences resulted in significant differences in live weight, cumulative weight gain, cumulative intake, feed conversion ratio, average daily gain, European production efficiency factor and protein efficiency ratio. However, these differences were not caused by the type of inorganic phosphate supplement used, but by the inclusion levels of these phosphates and this was regardless of the method of incorporation into the diet. Diets that received P supplementation based on the diets amount of total phosphorus were negatively affected and this is backed up by the liveability results that indicated possible P deprivation in the final week of the trial. When assessing broiler carcass characteristics and meat quality, the breast portion weight and breast and thigh

pH were not affected by the treatment differences, whereas the wing, thigh and drumstick portion weights and the breast colour CIE-Lab measurements (L*, a* and b*), as well as the chroma values, were influenced by treatments. After closer deliberation it was concluded that the dietary treatments were not the cause of the portion weight differences, but rather the cutting of the portions. Breast colour was all found to fall within the range that is considered normal despite the differences between the treatments. Breast hue angle was not influenced by the dietary treatment differences.

Weights of the spleen, liver and gizzard were significantly different; however, when expressed relevant to the bird's body weight, the differences were lost. Expressing the weights relative to body weight removes any differences that may have been caused by variation in the bird's size and therefore organ weight. The spleen:bursa ratio reported no significant difference between the treatments and hence the treatments did not affect the birds immune status. Differences in bone breakage strength indicate that the different levels of P inclusion is important to bone development and was not influenced by the type of P supplement nor of the method of incorporation into the diet. Bone mineralisation revealed no difference in the bones calcium (Ca) and P content as well as the Ca:P ratio within the bone. The pH readings of the proventriculus, duodenum, jejunum and ileum were unaffected by the dietary treatments. However, the cecum showed differences in pH and may be a cause of increased unabsorbed substrate reaching the cecum allowing for greater fermentation.

It can, therefore, be concluded that defluorinated PA and defluorinated and desulfonated PA are viable inorganic phosphate sources which can be used within broiler diets to meet the birds P requirements. The PA's reported no adverse effects on the birds' welfare nor production potential, provided P inclusion levels were sufficient. Further research is recommended to obtain the best inclusion level of PA such that the requirements are met with the least amount of P excretion possible.

Addendum A

Table A-1 Mean (± standard error) live weights of birds reared from hatch to day 35 on diets with different phosphorus sources at different P inclusion levels which were mixed to the feed in one of two manners without the control diet.

Treatment	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35
aP-DF-G	42.00 ± 1.07	160.38^{a} ± 1.98	397.30^{a} ± 10.70	863.75^{a} ± 20.02	1446.58^{a} ± 17.81	2190.54^{a} ± 36.97
aP -DF-L	41.94 ± 0.62	146.03^{bc} ± 3.55	354.83^{ab} ± 9.76	792.04^{ab} ± 17.10	1338.43^{ab} ± 25.98	2040.78^{a} ± 36.23
tP-DF-G	42.22 ± 0.51	134.90^{cd} ± 3.35	319.86^{b} ± 19.60	665.76^{cd} ± 12.68	1067.27^{c} ± 25.17	1584.98^{b} ± 41.66
tP -DF-L	42.97 ± 0.62	143.01^{bcd} ± 2.56	305.76^{b} ± 14.17	653.22^{d} ± 20.77	1125.18^{c} ± 31.81	1647.70^{b} ± 47.68
aP -DFS-G	41.66 ± 0.80	151.39^{ab} ± 2.83	355.27^{ab} ± 16.10	784.21^{ab} ± 27.06	1348.40^{ab} ± 30.91	2059.38^{a} ± 54.33
aP -DFS-L	42.60 ± 0.54	145.60^{bc} ± 1.97	342.22^{ab} ± 20.42	761.42^{bc} ± 34.96	1290.14^{b} ± 43.96	1968.54^{a} ± 72.49
tP -DFS-G	42.47 ± 0.62	130.63^{d} ± 2.96	292.65^{b} ± 18.50	602.31^{d} ± 14.46	979.60^{c} ± 34.20	1515.17^{b} ± 52.22
tP -DFS-L	43.07 ± 0.21	139.95^{bcd} ± 2.10	295.49^{b} ± 5.96	661.76^{cd} ± 16.23	1112.08^{c} ± 39.49	1625.49^{b} ± 42.66
p-value[#]	0.775	<0.01	<0.01	<0.01	<0.01	<0.01

^{a,b} means within columns that have different superscripts differ significantly ($p \leq 0.05$)
aP-DF-G: Formulated for available phosphorus using de-fluorinated PA, mixed with the grains.
aP-DF-L: Formulated for available phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
tP-DF-G: Formulated for total phosphorus using de-fluorinated PA, mixed with the grains.
tP-DF-L: Formulated for total phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
aP-DFS-G: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
aP-DFS-L: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.
tP-DFS-G: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
tP-DFS-L: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.
[#] p-value of the respective statistical analysis after the removal of the control diet due to potential differences caused by an increase in sodium at the start of the grower feeding period. Refer to section 4.5.4, page 79 for further explanations.

Table A-2 Mean (± standard error) cumulative weight gain of birds reared from hatch to day 35 on diets with different phosphorus sources at different P inclusion levels which were mixed to the feed in one of two manners without the control diet.

Treatment	Day 0-7	Day 0-14	Day 0-21	Day 0-28	Day 0-35
aP-DF-G	118.38^{a} ± 1.93	355.30^{a} ± 10.07	821.75^{a} ± 19.40	1404.58^{a} ± 18.06	2148.20^{a} ± 30.43
aP-DF-L	104.10^{bc} ± 3.43	287.11^{ab} ± 10.03	702.08^{ab} ± 16.10	1303.60^{a} ± 30.51	1980.55^{a} ± 40.47
tP-DF-G	91.85^{cd} ± 3.36	275.90^{b} ± 19.55	620.72^{cd} ± 14.30	1032.99^{bc} ± 25.10	1418.78^{b} ± 27.27
tP-DF-L	98.85^{bcd} ± 3.36	263.17^{b} ± 4.47	605.84^{d} ± 18.48	1089.62^{b} ± 37.17	1578.22^{b} ± 68.51
aP-DFS-G	108.72^{ab} ± 3.43	314.32^{ab} ± 15.46	743.27^{ab} ± 26.40	1307.46^{a} ± 30.14	2004.45^{a} ± 51.45
aP-DFS-L	103.00^{bc} ± 1.91	299.62^{ab} ± 20.18	718.20^{bc} ± 35.27	1257.28^{a} ± 36.88	1922.86^{a} ± 59.45
tP-DFS-G	88.16^{d} ± 3.23	250.18^{b} ± 18.28	555.98^{d} ± 14.98	938.27^{c} ± 36.06	1416.21^{b} ± 42.88
tP-DFS-L	97.17^{bcd} ± 1.80	254.37^{b} ± 6.62	620.64^{cd} ± 17.18	$1066.0b^{c}$ ± 35.28	1566.51^{b} ± 47.38
p-value[#]	<0.01	<0.01	<0.01	<0.01	<0.01

^{a,b} means within columns that have different superscripts differ significantly ($p \leq 0.05$)
aP-DF-G: Formulated for available phosphorus using de-fluorinated PA, mixed with the grains.
aP-DF-L: Formulated for available phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
tP-DF-G: Formulated for total phosphorus using de-fluorinated PA, mixed with the grains.
tP-DF-L: Formulated for total phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
aP-DFS-G: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
aP-DFS-L: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.
tP-DFS-G: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
tP-DFS-L: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.
[#] p-value of the respective statistical analysis after the removal of the control diet due to potential differences caused by an increase in sodium at the start of the grower feeding period. Refer to section 4.5.4, page 79 for further explanations.

Table A-3 Mean (± standard error) cumulative intake of birds reared from hatch to day 35 on diets with different phosphorus sources at different P inclusion levels which were mixed to the feed in one of two manners without the control diet.

Treatment	Day 0-7	Day 0-14	Day 0-21	Day 0-28	Day 0-35
aP-DF-G	151.27 ± 3.70	372.53 ± 11.22	1138.77^{a} ± 40.92	1997.92^{a} ± 40.66	3143.19^{a} ± 77.32
aP-DF-L	146.07 ± 3.12	338.27 ± 5.89	1091.70^{ab} ± 16.65	1956.57^{a} ± 30.39	3032.64^{a} ± 66.29
tP-DF-G	141.17 ± 7.48	332.00 ± 17.63	967.36^{abc} ± 30.60	1661.49^{bc} ± 28.89	2242.41^{b} ± 74.97
tP-DF-L	142.22 ± 8.34	325.51 ± 17.57	947.37^{bc} ± 24.29	1673.32^{bc} ± 40.40	2383.38^{b} ± 63.16
aP-DFS-G	141.78 ± 6.35	337.03 ± 11.26	1073.03^{ab} ± 21.94	1919.35^{a} ± 36.82	3019.67^{a} ± 89.52
aP-DFS-L	143.15 ± 4.84	325.08 ± 18.02	1028.72^{abc} ± 64.45	1850.05^{ab} ± 75.41	2877.85^{a} ± 34.84
tP-DFS-G	130.53 ± 6.77	284.03 ± 25.05	884.62^{c} ± 44.49	1553.02^{c} ± 60.15	2242.43^{b} ± 96.95
tP-DFS-L	142.75 ± 6.21	305.67 ± 27.96	970.44^{abc} ± 31.42	1679.33^{bc} ± 65.10	2450.90^{b} ± 96.49
p-value[#]	0.534	0.079	<0.01	<0.01	<0.01

[a,b] means within columns that have different superscripts differ significantly ($p \leq 0.05$)

Con: Control diet formulated for available phosphorus, using mono-dicalcium phosphate, mixed with the grains

aP-DF-G: Formulated for available phosphorus using de-fluorinated PA, mixed with the grains.

aP-DF-L: Formulated for available phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.

tP-DF-G: Formulated for total phosphorus using de-fluorinated PA, mixed with the grains.

tP-DF-L: Formulated for total phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.

aP-DFS-G: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.

aP-DFS-L: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.

tP-DFS-G: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.

tP-DFS-L: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.

[#] p-value of the respective statistical analysis after the removal of the control diet due to potential differences caused by an increase in sodium at the start of the grower feeding period. Refer to section 4.5.4, page 79 for further explanations.

Table A-4 Mean (± standard error) feed conversion ratios of birds reared from hatch to day 35 on diets with different phosphorus sources at different P inclusion levels which were mixed to the feed in one of two manners without the control diet.

Treatment	Day 0-7	Day 0-14	Day 0-21	Day 0-28	Day 0-35
aP-DF-G	1.28^{b} ± 0.03	1.05 ± 0.02	1.38^{b} ± 0.02	1.42^{d} ± 0.03	1.46 ± 0.02
aP-DF-L	1.41^{ab} ± 0.05	1.09 ± 0.04	1.47^{ab} ± 0.03	1.50^{bcd} ± 0.02	1.53 ± 0.02
tP-DF-G	1.54^{a} ± 0.05	1.21 ± 0.05	1.56^{a} ± 0.03	1.61^{ab} ± 0.01	1.58 ± 0.04
tP-DF-L	1.43^{ab} ± 0.05	1.24 ± 0.09	1.57^{a} ± 0.04	1.54^{abcd} ± 0.04	1.52 ± 0.04
aP-DFS-G	1.30^{b} ± 0.04	1.08 ± 0.04	1.45^{ab} ± 0.03	1.47^{cd} ± 0.01	1.51 ± 0.01
aP-DFS-L	1.39^{ab} ± 0.05	1.10 ± 0.06	1.43^{ab} ± 0.04	1.47^{bcd} ± 0.05	1.49 ± 0.04
tP-DFS-G	1.48^{ab} ± 0.03	1.13 ± 0.06	1.59^{a} ± 0.05	1.66^{a} ± 0.01	1.58 ± 0.04
tP-DFS-L	1.47^{ab} ± 0.08	1.20 ± 0.09	1.56^{a} ± 0.02	1.57^{abc} ± 0.03	1.57 ± 0.04
p-value[#]	<0.01	0.247	<0.01	<0.01	0.118

[a,b] means within columns that have different superscripts differ significantly ($p \leq 0.05$)
Con: Control diet formulated for available phosphorus, using mono-dicalcium phosphate, mixed with the grains
aP-DF-G: Formulated for available phosphorus using de-fluorinated PA, mixed with the grains.
aP-DF-L: Formulated for available phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
tP-DF-G: Formulated for total phosphorus using de-fluorinated PA, mixed with the grains.
tP-DF-L: Formulated for total phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
aP-DFS-G: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
aP-DFS-L: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.
tP-DFS-G: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
tP-DFS-L: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.
[#] p-value of the respective statistical analysis after the removal of the control diet due to potential differences caused by an increase in sodium at the start of the grower feeding period. Refer to section 4.5.4, page 79 for further explanations.

Table A-5 Mean (± standard error) average daily gain (ADG), European production efficiency ratio (EPER), protein efficiency ratio (PER) and liveability of birds reared from hatch to day 35 on diets formulated for total or available phosphorus, using phosphoric acid as the phosphorus source and different mixing sequences without the control diet.

Treatment	ADG (g)	EPEF	PER	Liveability (%)
aP-DF-G	62.59^{a} ± 1.06	428.29^{a} ± 8.19	3.32^{a} ± 0.05	100.00^{a} ± 0.00
aP-DF-L	58.31^{a} ± 1.04	368.19^{b} ± 10.68	3.24^{ab} ± 0.04	96.67^{ab} ± 2.11
tP-DF-G	45.29^{b} ± 1.19	236.13^{e} ± 8.70	2.90^{de} ± 0.06	83.33^{b} ± 6.15
tP-DF-L	47.08^{b} ± 1.32	292.61^{c} ± 21.65	3.09^{bc} ± 0.08	93.33^{ab} ± 3.33
aP-DFS-G	58.84^{a} ± 1.55	384.77^{b} ± 14.47	3.36^{a} ± 0.02	98.33^{ab} ± 1.67
aP-DFS-L	56.24^{a} ± 2.07	364.05^{b} ± 13.18	3.16^{bc} ± 0.06	96.67^{ab} ± 2.11
tP-DFS-G	43.29^{b} ± 1.49	244.92^{de} ± 24.50	3.01^{cde} ± 0.06	90.00^{ab} ± 5.16
tP-DFS-L	46.44^{b} ± 1.22	278.33^{cd} ± 13.97	3.04^{cd} ± 0.08	93.33^{ab} ± 2.11
p-value[#]	<0.01	<0.01	<0.01	0.032

[a,b] means within columns that have different superscripts differ significantly ($p \leq 0.05$)
Con: Control diet formulated for available phosphorus, using mono-dicalcium phosphate, mixed with the grains
aP-DF-G: Formulated for available phosphorus using de-fluorinated PA, mixed with the grains.
aP-DF-L: Formulated for available phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
tP-DF-G: Formulated for total phosphorus using de-fluorinated PA, mixed with the grains.
tP-DF-L: Formulated for total phosphorus using de-fluorinated PA, mixed in last at the end of the mixing process.
aP-DFS-G: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
aP-DFS-L: Formulated for available phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.
tP-DFS-G: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed with the grains.
tP-DFS-L: Formulated for total phosphorus using de-fluorinated and de-sulfonated PA, mixed in last at the end of the mixing process.
[#] p-value of the respective statistical analysis after the removal of the control diet due to potential differences caused by an increase in sodium at the start of the grower feeding period. Refer to section 4.5.4, page 79 for further explanations.